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Research Article

# *IN VITRO* GROWTH INHIBITORY EFFECT OF ZnO NANOPARTICLES ON HUMAN LIVER CANCER CELL LINES (Huh7)

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**ABSTRACT**

**Objective:** The objective of the study was to access the anticancer activity of the biosynthesized ZnO nanoparticles against Huh7 liver cancer cell lines.

**Methods:** The study was carried *in vitro* using Huh7 cell lines. The ZnO nanoparticles (ZnO NPs) were synthesized using *Luffa acutangula* peel extract and subjected to characterization by X-ray powder diffraction and transmission electron microscopy. The Huh7 cell lines were treated with ZnO NPs and done 3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide assay. For live and dead assay, the cell lines treated with ZnO NPs were subjected to acridine orange/ethidium (AO/ET) bromide assay.

**Results:** The ZnO NPs synthesized show spherical structure with 10–20 nm size. The 50% of Huh7 proliferation were inhibited at the concentration ($IC_{50}$) of 40 µg/ml. The AO/ET assay shows compact nucleus and fine cytoplasmic morphology in control cells and apoptotic stage in treated cells

**Conclusion:** This study suggests that ZnO NPs can be prepared in environment-friendly method using aqueous extract of *L. acutangula* and can be used in cancer treatment effectively.

**Keywords:** Nanoparticles, Eco-friendly synthesis, Electron microscopy, Huh7 cell lines, Anticancer activity, Cellular staining.

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## INTRODUCTION

The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Metal nanoparticles have tremendous applications in the area of catalysis photoelectronics and diagnostic biological probes and also in display devices [1]. The biological synthesis of nanoparticles less time and technology, cost effective and causes no health hazards when compared to physical and chemical synthesis of metal nanoparticles [2]. They are efficacious as they are done in single-pot reactions, without the need for additional surfactants and capping agents [3]. Now, metal nanoparticles got extensive attraction in biological applications due to their physiochemical properties [4]. Zinc oxide nanoparticles (ZnO NPs) are popularly employed in various fields due to their peculiar physical and chemical properties [5]. Cancer is an abnormal growth of tissue or cells exhibiting uncontrolled division autonomously resulting in a progressive increase in the number of cell divisions [6]. There is an increasing demand for anticancer therapy [7]. *In vitro* cytotoxicity testing procedures reduce the use of laboratory animals [8], and hence, the use of cultured tissues and cells has increased [9]. The use of chemotherapy to treat the cancer patients has its limitation due to its low specificity and also restriction toward its dosage level. To overcome these limitations, an alternate way of therapies and drugs is essential to treat the cancer. Nowadays, eco-friendly based nanoparticles are attempted by the researchers as an alternate to control the cancerous cell growth [10]. Nanomaterials are expected hopefully to revolutionize cancer diagnosis and therapy [11]. Huh-7 cells is a well-established and differentiated hepatocyte-derived cellular carcinoma cell line that can be propagated in a chemically defined medium containing trace amounts of selenium in place of serum [12]. The current study was aimed to investigate the antiproliferative effect of ZnO NPs synthesized using *Luffa acutangula* peel extract.

## MATERIALS AND METHODS

### Preparation of *L. acutangula* peel extract

*L. acutangula* was washed thoroughly using double distilled water and removed the peel. The peel was shade dried and was blended into fine powder. Added 100 ml of double distilled water to 10 g of peel powder and boiled at 100°C for 60 min. Cooled and filtered the boiled sample using Whatman No:1 filter paper. The aqueous extract thus obtained was used for further studies.

### Synthesis of ZnO NPs

ZnO NPs were prepared by adding 50 ml of the aqueous extract of *L. acutangula* peel to 5 g of zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$]. Following the addition, the mixture was kept in magnetic stirrer for drying. The dried sample was transferred to the crucible and calcined at 400°C for 2 h to obtain ZnO nanopowder.

### Characterization of ZnO NPs

The prepared ZnO NPs were then subjected to characterization by X-ray diffraction analysis and transmission electron microscopic analysis.

### Cellular cytotoxicity studies

The cellular cytotoxicity studies were achieved by the 3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The Huh-7 cells were seeded in 96-well plates ($1 \times 10^4$ cells/well) and allowed to attach overnight. The next day morning, old media were aspirated with a new fresh medium which was presented with different concentrations of ZnO NPs (0–100 µg). The plate was placed into an incubator for 8 h. After incubation, MTT solution was added and kept under dark condition inside the incubator for 4 h. After 4 h, the supernatant was removed and purple color formazan was added. The plate was then read at 595 nm in ELISA plate reader [13]. The optical density was used to calculate the cellular cytotoxicity of ZnO NPs in comparison with control cells.

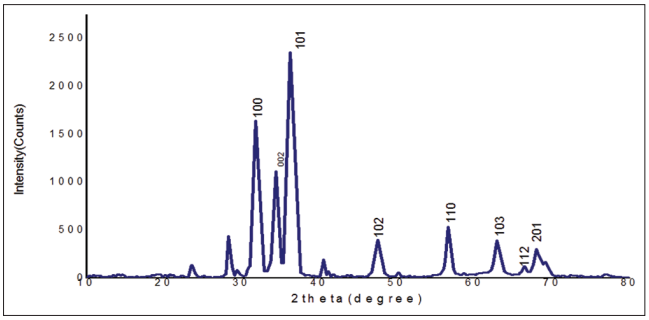


**Fig. 1: X-ray powder diffraction pattern of ZnO NPs**

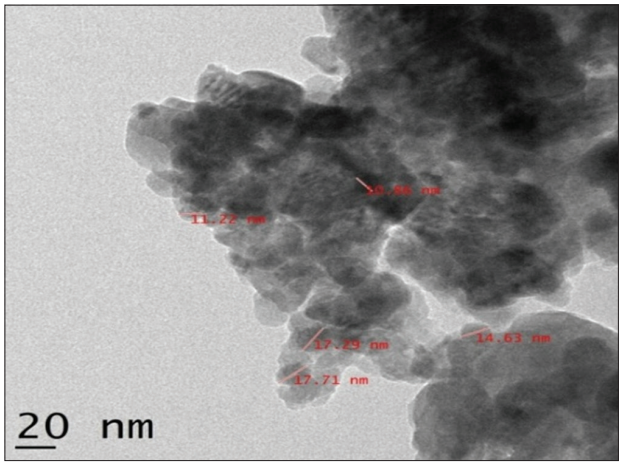


**Fig. 2: Transmission electron microscopy image of ZnO NPs**

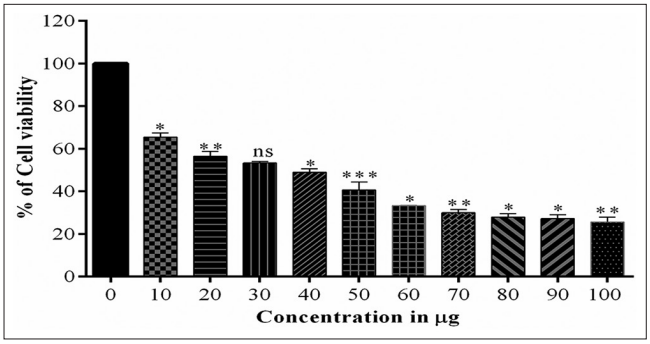


**Fig. 3: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay of ZnO-treated Huh7 cell**

**Acridine orange/ethidium bromide (AO/ETBR) staining**

For the live and dead assay, dual staining (AO/ETBR – 10 mg/ml) was used. Huh-7 cells were seeded in 6-well plates. After reaching 80% confluence, the cells were treated with 40 µg/ml of ZnO NPs and incubated. The cells without treatment act as control cells. The staining solution was added to control well and treated well. The plate was kept under a dark condition for 30 min [14]. The green filter and red filter were used to capture the cells morphology.

## RESULTS AND DISCUSSION

**X-ray diffraction studies**

The synthesized zinc oxide nanoparticles were subjected to X-ray diffraction studies and X-ray diffraction pattern was obtained (Fig. 1). From this figure, the peaks from (100), (002), (101), (102), (110), (103), (112), and (201) planes in hexagonal sort of ZnO NP were in brilliant record with the estimations of standard card (JCPDS NO=39–1451 universal place for diffraction information). Similar results were reported by Selvakumari *et al*. [15]. The peak positions at 28.328° and 40.511° may attribute to the compound in the peel extract. The sharp peak pattern explains that the nanoparticles were crystalline in nature. Furthermore, the grain size of the nanoparticles was found to be 21 nm as calculated by Debye-Scherrer equation.

**Transmission electron microscopic analysis**

The nanoparticles of size 10–20 nm were observed under transmission electron microscope (Fig 2). The results obtained were similar to the reports of Ratney and David [16].

**Anticancer activity studies**

*MTT assay*

The antiproliferative activity of zinc oxide nanoparticles was examined using MTT assay on human liver cancer cell line Huh7. The studies show that after 8 h of incubation with MTT solution, the $IC_{50}$ value was found to be 40 µg/ml (Fig. 3). The cytotoxic study of ZnO nanoparticles against breast cancer cell line (MCF7) by Moghaddam *et al*. [17] reported $IC_{50}$ as 121 µg/ml. Furthermore, Lingaraju *et al*. [18] reported the cytotoxicity activity of the green synthesized nanoparticles with $IC_{50}$ of 383 and 329 µg/ml against A549 and HepG2 cancer cell lines. The mechanism for the observed cytotoxic effects may probably be due to cell cycle arrest followed by apoptosis [19].

**Live and dead assay by AO/ETBR staining**

The cells were treated with nanoparticles of 40 µg/ml concentration (treated) and without nanoparticles (control). The fluorescence emission captured by fluorescence microscopy at ×20 shows compact nucleus and fine cytoplasmic morphology in control cells and apoptotic stage in treated cells (Fig. 4).

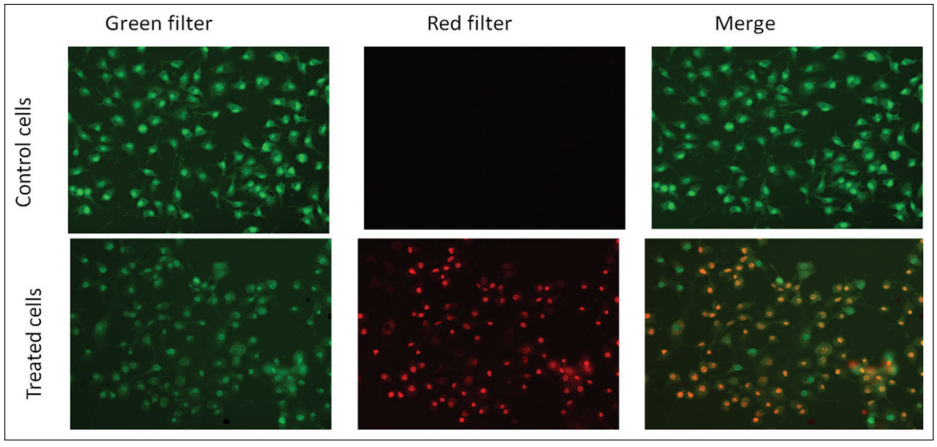


**Fig. 4: Cytoplasmic morphology of ZnO NPs treated and untreated Huh7 cells**

## CONCLUSION

The present study reveals that the eco-friendly synthesis of ZnO NPs using aqueous extract of *L. acutangula* peel is cost effective and simple method. The synthesized nanoparticles were characterized by X-ray powder diffraction and transmission electron microscopy analysis which confirmed their shape as spherical and crystalline in nature. Furthermore, the synthesized ZnO NPs showed the antiproliferative effect on Huh7 cancer cell lines at the $IC_{50}$ value of 40 µg/ml. Thus, ZnO NPs prepared in trouble less method using aqueous extract of *L. acutangula* can be used in cancer treatment.

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## AUTHORS' CONTRIBUTIONS

Ananthalakshmi R has designed the project, carried out the experiments, and analyzed them. Xavier Rajarathinam S. R supervised the research and helped in manuscript editing and Mohammed Sadiq. A has guided the research.

## CONFLICTS OF INTEREST

All authors have no conflicts of interest to report.

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