In vitro Cytotoxicity Studies of Zn (Zinc) Nanoparticles Synthesized from Abutilon indicum L. against Human Cervical Cancer (HeLa) Cell Lines

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ABSTRACT
Background: The Zn nanoparticles synthesized from the plant sources are ecofriendly and are potent anticancer agents. Objective: The objective of the present work was to evaluate In vitro cytotoxic activity of Zn nanoparticles green synthesized from Abutilon indicum extract against HeLa cell lines (cervical cancer). Methods: The aqueous extract is prepared by cold extraction (maceration) using water as a solvent. Phytochemical analysis was done by using the standard procedures. Aqueous extract of Abutilon indicum was used for synthesis of Zn nanoparticles. The nanoparticles were characterized by UV-Visible spectrometry and Scanning electron microscopy (SEM) techniques. In vitro cytotoxicity studies of Zn nanoparticles were done by MTT assay using HeLa cell lines. Results: The preliminary phytochemical results revealed that the aqueous extract of Abutilon indicum contains broad spectrum of secondary metabolites like Tannins, Saponins, Glycosides, Flavonoids, Anthroquinones, Terpenoids and Steroids. The U.V spectrophotometric analysis of Zn nanoparticles displayed maximum absorption at 270 nm and scanning electron microscopic studies showed that the nanoparticles size ranges from 50-500 nm. The MTT assay results revealed that the of Zn nanoparticles exhibits potent cytotoxicity against HeLa cell lines with IC50 value of 45.82 µg/ml. Conclusion: Thus the present study concludes that Zn nanoparticles can be used as a potent drug in alternative therapy for treating the cervical cancer patients. Key words: Abutilon indicum, Zn nanoparticles, MTT assay, Cervical cancer, Cytotoxicity

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INTRODUCTION

Nanoparticles are minute particles with dimension in 1-100 nm large, whose size is comparable to the biological molecules with its new and enhanced size-dependent properties compared to its bulk material, they penetrate more than larger substances and ideally interact with cellular structures.1-3 These nanoparticles can be synthesized by chemical processes like pyrolysis, hydrothermal method, chemical precipitation etc. But these chemical processes cause pollution and are costly practices. Synthesis of metal oxide nanoparticles can be done using biological materials such as microbes and plants. Synthesis of nanoparticles using microorganism involves lengthy process of maintaining microbial cultures, intracellular synthesis and multiple purification steps. However using “green” methods in the synthesis of Zinc nanoparticles has increasingly become a topic of interest. Nanoparticles in recent days have revolutionized the modern medicinal practices because of their potential activities in treatment of various diseases.4 Plants have been used from centuries to treat various human diseases. Herbal drugs as compared to synthetic drugs have no or lesser side effects and are less expensive. Since plant mediated synthesis is easy and safe with one-step protocol and don’t involve the use of harsh solvents or surfactant as the reducing agents, studies have suggested their use to be more ideal and compatible for their use in nanomedicine because of their stability in various biological media. The biomolecules present in plants mediates the synthesis of nanoparticles and also stabilize the nanoparticles formed with desired size and shape as well play a role in reducing the ions to the nanosize, and in the capping of nanoparticles.4 Numerous factors such as temperature, pH, concentration of extracts, concentration of raw material, etc. influence the reduction process of metal ions into the metal nanoparticles.4 Cancer is a class of disease where abnormal cells proliferate uncontrollably, producing malignant tumors that invade surrounding healthy tissue. Cervical cancers are carcinomas of squamous cell, arising in the squamous (flattened) epithelial cells that line the cervix. Due to serious side effects of currently available cancer treatments—chemotherapy and radiation therapy, death rate is high. Studies have showed that plant derived nanoparticles can be used to treat cancer patients in the near future with minimum side effects. The Plant based Zn nanoparticles were used because of their inbuilt ability to penetrate tissue and cells and interact with the cancerous cells.4 This property of Zn-nanoparticles can be further engineered by functionalization with target proteins or chemical groups and rendering them benign to normal cells while retaining their cancer targeting and killing properties.6-11 Studies demonstrate that cytotoxic property of Zn nanoparticles depends on their size i.e-smaller nanoparticles exhibit greater toxicity.12,13 The surface charge nature of Zinc nanoparticles is typically due to neutral hydroxyl groups attached to their surface.14,15 Also at lower pH Zn-nanoparticles gain positive charge from the environment, which then interact with negatively charged phospholipids on the membrane of cancer cells, thereby promoting cellular uptake, phagocytosis and cytotoxicity.16-18 Studies have showed that Zn nanoparticles exposure at particular concentration induces the production of various pro-inflammatory cytokines that elicits Th1-mediated immune response which inturnenhances tumor cell killing through production of TNF-α (Tumor Necrosis Factor).19-21 A. indicum generally known as Country Mallow belongs to the family Malvaceae. It is spread throughout the world, available in tropical and subtropical areas including India. It is a perennial herb, 1-3 m tall, with a woody perennial stem. Its leaves are simple, opposite, and entire. The flowers are yellow, with five petals. The fruits are dry, and covered with a white pubescence. The plant is used in traditional medicine for various purposes including as an antitumor, antibacterial, and anti-inflammatory agent.22-25
and used traditionally for the treatment of various ailments such as chest infection, urethritis, stranguery, haematuria, leprosy, ulcer, toothache, inflammation of bladder, piles, laxative, in chronic cystitis, gleet and gonorrhea, arthritis, seizures and in liver protection. There are limited numbers of reports available on the cytotoxic effect of Zn nanoparticles against cervical cancer, so the present study aims at profiling the same.

MATERIALS AND METHODS

Preparation of leaf extract
Fresh leaves were collected from *Abutilon indicum* plants in HSR Layout, Bangalore and it was identified and authenticated by taxonomist. A voucher specimen was deposited in The Oxford College of Pharmacy, Bangalore. The leaves were washed several times with water to remove the dust particles and then shade dried to remove the remaining moisture. The dried leaves were coarsely powdered and then placed in sterile distilled water in a conical flask so that all the crushed leaves were properly dipped in water. The conical flask was placed on a shaker for the cold extraction for 12-15 hrs. The extract was filtered using muslin cloth and the filtrate was poured into petriplate & dried using dessicator to form a thick jelly like extract.

Preliminary Phytochemical Analysis
Phytochemical analysis was performed using standard procedures by Harborne JB, 1998.

Synthesis of Zn Nanoparticles
Zinc acetate dihydrate and sodium hydroxide were used as the precursor material. The dried aqueous extract was dissolved in respective solvent, 30 ml was prepared. Three sets of conical flasks with 50 ml distilled water each were prepared and labeled as 0.25 ml, 0.5 ml and 1 ml respectively. 0.02 M aqueous Zinc acetate dihydrate (0.219 gm) was added to each of the three flasks under vigorous stirring. After 10 min stirring aqueous leaf extract of *Abutilon indicum* were introduced into the above solution with the concentration of 0.25 ml, 0.5 ml, 1 ml respectively. The Zn nanoparticles synthesized were observed by change of colour to white precipitate, the precipitate was dried and used for UV spectrophotometer analysis. The Zn nanoparticles synthesized were observed by change of colour to white precipitate, the precipitate was dried and used for UV spectrophotometer analysis. The Zn nanoparticles synthesized were observed by change of colour to white precipitate, the precipitate was dried and used for UV spectrophotometer analysis.

Charaterization of Zn Nanoparticles
The Zn nanoparticles were characterized by using a UV-Vis spectrometer, the nanoparticle suspension was prepared in deionized water; the supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line.

% Growth Inhibition = 100 - \left( \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \right) \times 100

RESULTS
The preliminary phytochemical analysis inferred the presence of different classes of phytoconstituents which are depicted in Table 1.

Characterization of Zn Nanoparticles
The Zn nanoparticles synthesized were observed by change of colour to white precipitate, the precipitate was dried and used for UV spectrophotometer analysis Graph 1 shows the UV spectrophotometer result with maximum absorption 270 nm.

SEM Analysis
The Scanning Electron Microscope (SEM) analysis was used to determine the structure of the reaction products that were formed. SEM
Table 1: Preliminary phytochemical analysis of aqueous extract of *Abutilon indicum*

| SL NO. | TEST     | INFERENCES |
|--------|----------|------------|
| 1      | Reducing Sugar | +          |
| 2      | Tannin    | +          |
| 3      | Alkaloid  | -          |
| 4      | Saponin   | +          |
| 5      | Glycosides| +          |
| 6      | Steroid   | +          |
| 7      | Flavanoids| +          |
| 8      | Anthroquinones| +     |
| 9      | Terpenoids| +          |

Table 2: MTT assay results showing cell viability (%) based on concentration

| SL.No. | Concentration (µg/ml) | Cell Viability (%) | Sample | Standard (5-FU) |
|--------|-----------------------|--------------------|--------|-----------------|
| 1      | 5                     | 89.540             | 70.126 |
| 2      | 10                    | 79.714             | 42.364 |
| 3      | 20                    | 69.889             | 37.562 |
| 4      | 30                    | 58.795             | 33.891 |
| 5      | 40                    | 54.358             | 30.456 |
| 6      | 50                    | 50.396             | 25.755 |
| Control| -                     | 100                | -      |

**DISCUSSION**

*A. indicum* is an important medicinal plant used traditionally to treat many human ailments. In the present study the preliminary phytochemical analysis of *A. indicum* revealed the presence of broad range...
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The Zn nanoparticles synthesized using the Seaweed Ulva lactuca were synthesized by using zinc acetate dihydrate as the precursor material with the plant extracts. These Zn nanoparticles when subjected to cytotoxicity assay exhibited potential cytotoxicity against HeLa cell lines with IC50 of 45.82 μg/ml (Graph 3). The cytotoxicity was found to be in a dose dependent manner (Graph 2). These Zn nanoparticles may selectively kill the cancer cells by apoptosis induced by the production of reactive oxygen species (ROS) via p53 pathway. This ability of the Zn nanoparticles to inhibit the growth of cancer cell can be used in the treatment of cervical cancer.

CONCLUSION

The Zn nanoparticles synthesized using A. indicum, showed positive results for cytotoxic activity with an IC50 value of 45.82 μg/ml. These positive results confirmed the cytotoxic potential of the Zn nanoparticles against cervical cancer. Thus Zn nanoparticles can be used as a potent drug in alternative therapy for treating the cervical cancer patients in the near future. Further potential of cytotoxicity against cancer can be enhanced by In vivo studies in animal models and human volunteers.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATION USED

Zn: Zinc; HeLa: Henrietta Lacks; SEM: Scanning Electron Microscope; UV-Vis: Ultraviolet-Visible; MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; nm: Nanometer; Th1: TNF-a; Tumor Necrosis Factor; hrs: Hours; ml: Millilitre; M: Molar; gm: Gram; min: Minutes; KV: Kilovolts; DMEM: Dulbecco’s Modified Eagle’s Medium; EDTA: Ethylene Diamine Tetra Acetic acid; DMSO: Dimethyl Sulfoxide; FBS: Fetal Bovine Serum; IU/ml: International Units/millilitre; μg: Microgram; TPVG: Trypsin Phosphate Versene Glucose; cm: centimeter; mg: milligram; µl: microliter; OD: optical density; 5-FU: 5-Fluoro Uracil; IC: Inhibitory Concentration.

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PICTORIAL ABSTRACT

SUMMARY

• *Abutilon indicum* is a potent medicinal plant used in traditional medicine.
• The preliminary phytochemical analysis revealed the presence of broad spectrum of secondary metabolites.
• Zn nanoparticles synthesized from the aqueous extract of *A. indicum* showed strong cytotoxic activity against HeLa cell lines.

ABOUT AUTHORS

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