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# EVALUATION OF ANTICANCER POTENTIAL OF WITHANIA SOMNIFERA LOADED PLGA NANOFORMULATION ON MCF-7 CELL LINE

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## ABSTRACT

Cancer remains a major health challenge worldwide, with breast cancer being one of the most frequently diagnosed malignancies. Conventional chemotherapy is often associated with severe adverse effects and poor selectivity, which limits its clinical success. Herbal medicines have gained increasing attention due to their safety and therapeutic potential. Withania somnifera is a well-known medicinal plant possessing significant anticancer activity attributed to the presence of withanolides. However, its application is restricted by poor solubility and bioavailability. The present study was aimed to develop a PLGA-based nanoformulation of Withania somnifera extract and evaluate its apoptotic activity against MCF-7 breast cancer cell line. The nanoformulation was prepared using nanoprecipitation technique and characterized for particle size, zeta potential and entrapment efficiency. In-vitro cytotoxicity was evaluated using MTT assay. The nanoformulated extract exhibited enhanced cytotoxic and apoptotic activity compared to the crude extract. The findings suggest that PLGA-based nanoformulation of Withania somnifera may offer a potential nano-herbal strategy for further investigation in breast cancer management.

**KEYWORDS:** Withania somnifera; PLGA nanoparticles; Nanoformulation; MCF-7; Apoptosis.

## INTRODUCTION

Breast cancer is one of the leading causes of cancer-related deaths among women globally. Despite significant advancements in cancer therapy, conventional chemotherapeutic agents often suffer from drawbacks such as systemic toxicity, non-specific targeting and drug resistance. These limitations have encouraged the exploration of alternative therapeutic strategies.

Medicinal plants have been widely explored as potential sources of anticancer agents. Withania somnifera (Ashwagandha), belonging to the family Solanaceae, is traditionally used in Ayurvedic medicine and has been reported to possess anticancer, antioxidant and immunomodulatory activities.[1,2] The anticancer activity of Withania somnifera is mainly attributed to withanolides, particularly withaferin A, which induces apoptosis in cancer cells through mitochondrial pathways.[3,4]

However, poor solubility and low bioavailability limit the therapeutic efficacy of Withania somnifera. Nanotechnology-based drug delivery systems offer an effective approach to overcome these limitations. PLGA is a biodegradable and biocompatible polymer widely used in nano drug delivery.[5,6] Therefore, the present study focuses on the formulation of PLGA-based nanoformulation of Withania somnifera and evaluation of its apoptotic activity against MCF-7 breast cancer cell line.

## MATERIALS AND METHODS

### Plant Material and Extraction

Roots of Withania somnifera were procured from a local herbal supplier and authenticated by a pharmacognosy expert. The roots were washed thoroughly with distilled water to remove adhering dirt and impurities. The cleaned roots were shade-dried at room temperature for several days until a constant weight was obtained. The dried roots were then coarsely powdered using a mechanical grinder and passed through a sieve to obtain uniform particle size.

Approximately 100 g of the powdered material was subjected to hydro-alcoholic extraction using Soxhlet apparatus. A mixture of ethanol and distilled water was used as the extraction solvent. The extraction process was continued for several cycles until the solvent in the siphon tube became colorless, indicating complete extraction. The obtained extract was

filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was dried and stored in an airtight container at 4°C until further use.

**Preparation of PLGA Nanoformulation**

PLGA-based nanoparticles loaded with Withania somnifera extract were prepared using the nanoprecipitation method. Initially, a known quantity of PLGA polymer was dissolved in acetone to form the organic phase. The previously prepared Withania somnifera extract was added to this polymer solution and mixed thoroughly to ensure uniform dispersion.

Separately, an aqueous phase containing a stabilizer was prepared and maintained under continuous magnetic stirring. The organic phase was added dropwise into the aqueous phase using a syringe under constant stirring. The spontaneous diffusion of the organic solvent into the aqueous phase led to the formation of nanoparticles. Stirring was continued to allow complete evaporation of the organic solvent. The resulting nanoparticle suspension was collected and stored for further evaluation.

**Characterization of Nanoformulation**

The prepared PLGA nanoformulation was characterized to evaluate its physicochemical properties. Particle size and size distribution were determined to confirm that the nanoparticles were within the nano-range. Zeta potential was measured to assess the surface charge and stability of the nanoparticles. Entrapment efficiency was determined to evaluate the amount of Withania somnifera extract successfully encapsulated within the PLGA nanoparticles. All measurements were performed using standard analytical procedures.

**In-Vitro Cytotoxicity Study**

The in-vitro cytotoxic activity of the crude extract and PLGA nanoformulation was evaluated using MCF-7 human breast cancer cell line. The cells were cultured in suitable growth medium under controlled conditions of temperature and carbon dioxide. After attaining sufficient growth, the cells were seeded into multi-well plates and allowed to adhere.

The cells were treated with different concentrations of crude Withania somnifera extract and PLGA nanoformulation. Untreated cells served as control. After incubation for a specified period, cell viability was assessed using MTT assay. The MTT reagent was added to each well and incubated to allow the formation of formazan crystals by metabolically active cells. The crystals were dissolved using suitable solvent, and absorbance was measured using a

microplate reader. Cell viability was calculated and expressed as percentage relative to control.

**Evaluation of Apoptosis**

Apoptotic activity was evaluated by observing morphological changes in treated MCF-7 cells. After treatment with crude extract and nanoformulation, the cells were examined under an inverted microscope. Morphological features such as cell shrinkage, membrane blebbing, loss of cell integrity and nuclear condensation were considered indicative of apoptotic cell death. The observed changes were compared with control cells to confirm apoptotic induction by the nanoformulation.

## RESULTS AND DISCUSSION

**Characterization of PLGA nanoformulation of Withania somnifera**

| Parameter | Result(Mean ± SD) |
|---|---|
| Particle size (nm) | 185.4 ± 12.6 |
| Zeta potential (mV) | −21.8 ± 2.3 |
| Entrapment efficiency (%) | 72.6 ± 3.1 |

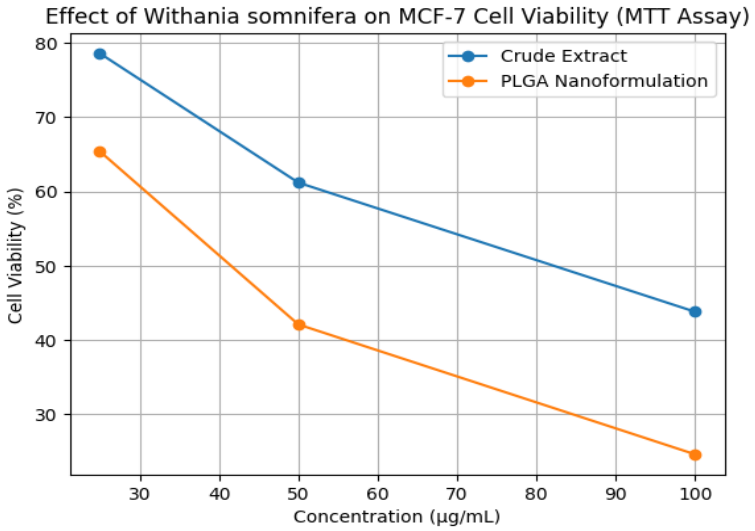
**Effect of Withania somnifera extract and PLGA nanoformulation on MCF-7 cell viability**

| Treatment | Concentration(µg/mL) | Cell Viability (%) |
|---|---|---|
| Control | 0 | 100 ± 2.1 |
| Crude extract | 50 | 61.2 ± 2.9 |
| Crude extract | 100 | 43.8 ± 3.1 |
| Nanoformulation | 50 | 42.1 ± 3.0 |
| Nanoformulation | 100 | 24.6 ± 2.5 |

All experiments were performed in triplicate and results were expressed as mean ± SD. Statistical significance was considered at $p < 0.05$.

The nanoformulation exhibited dose-dependent cytotoxicity against MCF-7 cells and showed greater reduction in cell viability compared to the crude extract. Morphological observations confirmed apoptotic changes in treated cells.

The graphical representation of MTT assay results demonstrated a dose-dependent decrease in cell viability in MCF-7 cells. The PLGA nanoformulation exhibited significantly higher cytotoxicity compared to the crude extract at equivalent concentrations, indicating enhanced anticancer activity due to nano-delivery.




The enhanced cytotoxic and apoptotic activity of the PLGA-based nanoformulation can be attributed to improved cellular uptake and sustained release of bioactive compounds.[13,14] The nano-size of the formulation facilitates better penetration into cancer cells, while PLGA provides controlled drug release and stability. The apoptotic effects observed in MCF-7 cells are consistent with previous reports on withanolide-induced apoptosis mediated through mitochondrial pathways.

## CONCLUSION

The present study successfully developed a PLGA-based nanoformulation of Withania somnifera with enhanced apoptotic activity against MCF-7 breast cancer cell line. The nanoformulation demonstrated superior cytotoxicity compared to the crude extract, indicating its potential as an effective nano-herbal anticancer approach. Further in-vivo studies are required to confirm its therapeutic efficacy.

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