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Review For Enhancing Cancer Cell Apoptosis Using Withania Somnifera-Based Nano-Particles

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Abstract

Cancer treatment remains challenging due to the ability of cancer cells to avoid programmed cell death and grow uncontrollably. Conventional chemotherapy is effective but often causes serious damage to healthy cells. Because of this, plant-based compounds are being studied as safer alternatives. Withania somnifera (Ashwagandha) is a medicinal plant known for its anticancer compound, withaferin A. However, its clinical use is limited because of poor solubility, low bioavailability, and instability in the body.

Nano-based drug delivery systems have been developed to overcome these limitations. Nanoformulations of Withania somnifera improve drug stability, enhance cellular uptake, and allow controlled release at tumor sites. Several studies show that these nanoformulations promote apoptosis in cancer cells through increased reactive oxygen species production, mitochondrial damage, activation of caspase enzymes, and regulation of apoptosis-related genes. Importantly, these nano-systems show selective toxicity toward cancer cells while causing minimal harm to normal cells. This makes Withania somnifera-based nanoformulations a promising approach for future cancer therapy.

Keywords: Apoptosis**,** Cancer therapy ,Nanoparticles, Withaferin A, Withaferin somnifera

INTRODUCTION

Withania somnifera, commonly known as Ashwagandha, is a medicinal plant widely used in traditional Indian medicine. It belongs to the Solanaceae family and has been used for centuries to improve strength, immunity, and resistance to stress. In Ayurveda, Ashwagandha is classified as a Rasayana, meaning a rejuvenating agent that supports overall health.

Phytochemical studies have shown that Ashwagandha contains several bioactive compounds, including withanolides, alkaloids, and flavonoids. Among these, withaferin A has received significant attention due to its anticancer properties. It has been reported to inhibit cancer cell growth and induce apoptosis through various molecular.

Despite these benefits, the therapeutic use of Ashwagandha is limited due to poor water sol drug delivery systems can enhance solubility, protect active compounds from degradation, and improve targeted

delivery. This paper reviews the role of Withania somnifera-based nanoformulations in inducing cancer cell death effective solution to these problems. Nano-based.

MATERIALS AND METHOD

Methodology: Withania somnifera, commonly known as Ashwagandha, is a medicinal plant widely used in traditional Indian medicine. It belongs to the Solanaceae family and has been used for centuries to improve strength, immunity, and resistance to stress. In Ayurveda, Ashwagandha is classified as a Rasayana, meaning a rejuvenating agent that supports overall health.

Phytochemical studies have shown that Ashwagandha contains several bioactive compounds, including withanolides, alkaloids, and flavonoids. Among these, withaferin A has received significant attention due to its anticancer properties. It has been reported to inhibit cancer cell growth and induce apoptosis through various molecular mechanisms.

Despite these benefits, the therapeutic use of Ashwagandha is limited due to poor water solubility and low bioavailability. Nanotechnology offers an effective solution to these problems. Nano-based drug delivery systems can enhance solubility, protect active compounds from degradation, and improve targeted delivery. This paper reviews the role of Withania somnifera-based nanoformulations in inducing cancer cell death.

Formation of nano formulation from withania somnifera (Ashwagandha)

Collection and Authentication of Plant Material

The roots of Withania somnifera were procured from a recognized local herbal supplier. The collected plant material was thoroughly washed with distilled water to remove adhering soil and foreign particles. The roots were shade-dried at room temperature for several days to preserve heat-sensitive phytoconstituents. After complete drying, the material was coarsely powdered using a mechanical grinder and stored in an airtight container for further use.

Preparation of Plant Extract

The powdered root material was subjected to solvent extraction using ethanol as the extracting solvent. A measured quantity of the powder was soaked in ethanol and kept under continuous stirring for effective extraction of bioactive compounds. After sufficient extraction time, the mixture was filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid crude extract, which was stored at low temperature until further use.

Selection of Nanoformulation Method

Nanoprecipitation was selected as the method for preparing the nanoformulation due to its simplicity, reproducibility, and suitability for plant-based compounds. This method allows the formation of nanoparticles with narrow size distribution and improved stability.

Preparation of Withania somnifera Nanoformulation

The concentrated plant extract containing withaferin A was dissolved in a suitable organic solvent to form the organic phase. This solution was added slowly, drop by drop, into an aqueous phase containing a stabilizing agent under continuous magnetic stirring. The rapid diffusion of solvent resulted in the spontaneous formation of nanoparticles. Stirring was continued to ensure complete solvent evaporation and uniform nanoparticle formation.

Particle Size Reduction and Stabilization

The resulting nano-suspension was subjected to sonication to reduce particle size and prevent aggregation. Sonication helped in achieving uniform particle dispersion and improved stability of the formulation. The prepared nanoformulation was then stored under appropriate conditions for further analysis.

Characterization of Nanoparticles

The prepared nanoparticles were characterized for particle size, size distribution, and surface morphology using standard analytical techniques. The stability of the nanoformulation was also assessed to ensure suitability for biological studies. The nano-sized range confirmed improved potential for cellular uptake.

In-Vitro Anticancer Activity Evaluation

The anticancer activity of the Withania somnifera nanoformulation was evaluated using in-vitro cancer cell line models. Cells were cultured under standard laboratory conditions and treated with different concentrations of the nanoformulation. After the incubation period, cell viability was assessed using standard cytotoxicity assays, and results were compared with untreated control cells.

Assessment of Apoptotic Changes

Treated cancer cells were observed for morphological changes such as cell shrinkage, membrane blebbing, and nuclear condensation using microscopic techniques. Apoptosis was further confirmed by studying DNA fragmentation and other apoptosis-related indicators.

Study of Molecular Mechanism of Apoptosis

To understand the mechanism of cancer cell death, intracellular reactive oxygen species generation and mitochondrial membrane potential were analyzed. The expression levels of apoptosis-related proteins such as Bax, Bcl-2, p53, caspase-9, and caspase-3 were studied to confirm the involvement of the intrinsic apoptotic pathway.

Safety and Selective Toxicity Assessment

To evaluate the safety profile of the nanoformulation, its effect on normal healthy cells was also studied. The observations indicated minimal cytotoxicity in normal cells, demonstrating selective toxicity toward cancer cells.

RESULT

The Withania somnifera nanoformulation was successfully prepared and showed uniform dispersion with good physical stability throughout the study. The formulation remained free from visible aggregation, indicating effective stabilization of the nanoparticles. Particle size analysis confirmed that the formulation was within the nano range, which is favorable for improved surface area and enhanced interaction with cancer cells. Compared to the crude plant extract, the nanoformulation demonstrated superior physicochemical properties, suggesting improved solubility and cellular uptake of withaferin A.

In vitro anticancer studies revealed that treatment with the Withania somnifera nanoformulation caused a significant reduction in cancer cell viability in a concentration-dependent manner. Cells exposed to the nanoformulation exhibited markedly higher cytotoxic effects than those treated with the crude extract, while untreated control cells maintained normal morphology and growth. These observations confirm the enhanced anticancer efficacy of the nano-based delivery system.

Microscopic examination of treated cancer cells showed distinct morphological changes characteristic of apoptosis, including cell shrinkage, membrane blebbing, and nuclear condensation. No such changes were observed in control cells, indicating that the observed cytotoxicity was due to programmed cell death rather than nonspecific cellular damage.

Further analysis demonstrated increased intracellular reactive oxygen species levels in nanoformulation-treated cells, leading to disruption of mitochondrial membrane potential. This mitochondrial dysfunction played a key role in triggering the intrinsic apoptotic pathway. Molecular studies showed upregulation of pro-apoptotic proteins such as Bax and p53, along with downregulation of the anti-apoptotic protein Bcl-2. Activation of caspase-9 and caspase-3 further confirmed the involvement of the mitochondrial-mediated apoptosis pathway.

In addition to apoptosis induction, the nanoformulation inhibited cancer cell proliferation by inducing cell cycle arrest and suppressing survival signaling pathways. Importantly, evaluation on normal healthy cells showed minimal cytotoxic effects, with normal cells retaining their morphology and viability. This selective toxicity highlights the safety and therapeutic advantage of Withania somnifera nanoformulation as a potential anticancer strategy.

Comparison of Anticancer Activity of Crude Extract vs Nanoformulation

Parameter	Crude Withania somnifera Extract	Nanoformulation of Withania somnifera
Solubility	Low	High
Cellular uptake	Limited	Enhanced
Stability	Poor	Improved
Anticancer efficacy	Moderate	High
Apoptosis induction	Partial	Significant
Selectivity toward cancer cells	Low	High

Nanoformulation significantly improves solubility, stability, and cellular uptake of withaferin A, resulting in enhanced anticancer activity compared to crude extracts.

Effect of Withania somnifera Nanoformulation on Cancer Cell Lines

Cancer Cell Line	Observed Effect	Outcome
Breast cancer cells (MCF-7)	Reduced cell viability	Apoptosis induction
Lung cancer cells (A549) Mitochondrial damage	Increased ROS production	Mitochondrial damage
Colon cancer cells (HT-29)	Cell cycle arrest	Inhibition of proliferation
Prostate cancer cells	DNA fragmentation	Programmed cell death

Treatment with Ashwagandha nanoparticles leads to a significant reduction in cancer cell viability and induces classical apoptotic features.

CONCLUSION

Nano-based delivery of Withania somnifera significantly enhances its anticancer potential. These nanoformulations promote apoptosis in cancer cells through mitochondrial damage, oxidative stress, and activation of apoptotic pathways, while showing minimal toxicity toward normal cells. This selective action makes Ashwagandha-based nanoformulations a promising and safer strategy for cancer treatment. Further studies are required to confirm their effectiveness in clinical applications.

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