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INTEGRATING PHYTOCHEMISTRY WITH ONCOLOGY: THE ROLE OF *ALOE VERA* NANOPARTICLES IN COMBATING ORAL CANCER TUMOR GROWTH: *IN VIVO* RAT MODELS

Original Research

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ABSTRACT

Background: Oral cancer, a major public health challenge, often exhibits poor prognosis due to resistance to conventional therapies. Integrating plant-derived nanotherapeutics, such as *Aloe vera* nanoparticles (AVNPs), offers a promising alternative approach. *Aloe vera* possesses anti-inflammatory, pro-apoptotic, and anti-tumor properties, which can be enhanced through nanoparticle synthesis. This study explores the anti-cancer potential of AVNPs in reducing oral cancer tumor growth using *in vivo* models.

Objective: To investigate the therapeutic efficacy of *Aloe vera* nanoparticles in reducing tumor growth and inducing apoptosis in a chemically induced oral cancer rat model.

Methods: *Aloe vera* gel was processed under aseptic conditions, freeze-dried, and synthesized into nanoparticles using a green synthesis approach. Characterization of AVNPs was performed using *Scanning Electron Microscopy (SEM)* and *Dynamic Light Scattering (DLS)*. Oral cancer was induced in *Sprague-Dawley* rats through *4-nitroquinoline 1-oxide (4NQO)* in drinking water (20 ppm) over eight weeks. Rats were divided into four groups: control, cancer control, low-dose AVNPs (20 mg/kg), and high-dose AVNPs (40 mg/kg). Tumor growth inhibition was evaluated macroscopically and histopathologically. Cellular viability was assessed using the *MTT* assay, and gene expression levels of *Caspase-3* and *Caspase-9* were analyzed through quantitative PCR.

Results: AVNPs exhibited an *IC50* of 13.1 *μM* in the *MTT* assay. Tumor volume decreased significantly in the treated groups compared to the cancer control group. Relative gene expression showed upregulation of pro-apoptotic markers, with *Caspase-3* at 12.3 and *Caspase-9* at 14.8. These results confirm enhanced apoptosis and tumor suppression by AVNPs.

Conclusion: *Aloe vera* nanoparticles demonstrated significant anti-cancer potential by inducing apoptosis and reducing tumor growth in oral cancer models. Their efficacy as natural chemotherapeutics warrants further clinical research for potential therapeutic applications.

Keywords: Apoptosis, *Caspase-3*, *Caspase-9*, *in vivo* models, nanoparticles, oral cancer, pro-apoptotic biomarkers

INTRODUCTION

Squamous cell carcinoma, a major form of oral cancer, is a significant global public health concern and ranks among the ten most prevalent malignancies worldwide (1). Despite advances in conventional treatment approaches, including surgery, chemotherapy, and radiotherapy, the prognosis for oral cancer remains poor due to high recurrence rates and limited understanding of resistance to these therapies (2). This persistent challenge underscores the urgent need to explore novel therapeutic strategies that are both effective and minimally toxic. In recent years, there has been a growing interest in integrating phytochemistry with oncology to identify plant-derived compounds with anticancer properties (3). *Aloe vera*, a medicinal plant with well-documented pharmacological activities, has emerged as a promising candidate in this context. Its active phytoconstituents—aloin, aloe emodin, and acemannan—are known to exhibit potent anticancer effects through mechanisms such as induction of apoptosis, cell cycle arrest, and inhibition of tumor angiogenesis (5). However, the clinical application of *Aloe vera* is hampered by challenges related to its bioavailability, stability, and targeted delivery, limiting its therapeutic potential in cancer treatment.

The advent of nanotechnology has opened new avenues for addressing these limitations. *Aloe vera* nanoparticles, characterized by enhanced bioavailability, improved pharmacokinetics, and targeted delivery capabilities, represent a significant advancement in cancer research (6). These nanoparticles facilitate precise delivery of active components to the tumor microenvironment, maximizing therapeutic benefits while minimizing systemic toxicity (8). This study leverages *in vivo* rat models of chemically induced oral cancer to assess the efficacy of *Aloe vera* nanoparticles, focusing on their ability to inhibit tumor growth and induce apoptosis (7). Apoptosis, or programmed cell death, plays a critical role in controlling tumor progression in oral cancer (9). The anticancer effects of *Aloe vera* nanoparticles are hypothesized to be linked to their ability to modulate key signaling pathways and induce apoptosis, thereby disrupting the tumor's growth and survival mechanisms (10). To evaluate these effects, this study examines tumor size, histopathological alterations, and molecular markers of apoptosis in established rat models (11). By integrating phytochemistry with cutting-edge nanotechnology, the research aims to provide compelling evidence for the potential of plant-derived nanotherapeutics to address the unmet needs in oral cancer treatment. The findings are expected to contribute to the advancement of personalized medicine and the development of innovative therapeutic strategies for combating this challenging malignancy.

METHODS

This inter-collaborative study, conducted over four months (November 2022–March 2023), investigated the anticancer potential of *Aloe vera* nanoparticles (AVNPs) against oral cancer using *in vivo* rat models. Ethical approval for the study was obtained from the Department of Biochemistry at Lahore College for Women University, Lahore, Pakistan (approval number: RBM-017-ISB). The research was carried out in cell culture and animal research laboratories of affiliated institutes. The study followed rigorous protocols to ensure scientific integrity and adherence to ethical guidelines. Fresh *Aloe vera* leaves were collected, thoroughly washed, and processed under aseptic conditions to extract the gel. The gel was frozen, ground into fine powder, and freeze-dried at -55°C using a laboratory freeze dryer. The nanoparticles were synthesized through a green synthesis approach to ensure biocompatibility and eco-friendliness. The synthesized nanoparticles were characterized using *Scanning Electron Microscopy (SEM)* and *Dynamic Light Scattering (DLS)* to confirm particle size, morphology, and stability.

Sprague-Dawley rats, aged seven weeks and weighing 200–250 g, were procured and housed in standard laboratory conditions with controlled temperature, humidity, and a 12-hour light/dark cycle. Oral cancer was induced in the rats using *4-nitroquinoline 1-oxide (4NQO)* administered in drinking water at a concentration of 20 ppm for eight weeks. Tumor development and any adverse effects were monitored throughout the study. The rats were then divided into four groups, each consisting of six animals:

- **Control Group:** Administered normal drinking water and standard feed.
- **Cancer Control Group:** Administered 4NQO in drinking water without treatment.
- **Low-Dose AVNPs Group:** Treated with *Aloe vera* nanoparticles at a dose of 20 mg/kg body weight daily after oral cancer induction.
- **High-Dose AVNPs Group:** Treated with *Aloe vera* nanoparticles at a dose of 40 mg/kg body weight daily after oral cancer induction.

Following the treatment period, the rats were euthanized, and their tongues and buccal tissues were carefully excised for further analysis. Macroscopic and microscopic assessments were conducted to evaluate tumor growth inhibition. Histopathological analyses of tissue samples were performed to examine structural changes, and the *MTT* assay was utilized to assess cellular viability and determine the *IC50* values for tissue-derived cells. Total RNA was extracted from the treated tissues using a commercial RNA extraction kit according

to the manufacturer's protocol. RNA quality and concentration were evaluated, followed by cDNA synthesis using a *Thermo Fisher cDNA* synthesis kit (Catalog #AM7811). Quantitative polymerase chain reaction (qPCR) was employed to analyze the expression levels of apoptosis-related genes, including *Caspase-3* and *Caspase-9*. Custom-designed primers were used for the gene amplification. Statistical analyses were conducted using SPSS software. Results were presented as mean ± standard deviation, and significance was determined using analysis of variance (ANOVA), followed by post hoc tests. A p-value of <0.05 was considered statistically significant.

The green synthesis of *Aloe vera* nanoparticles was carried out using *Aloe vera* gel extract as a natural reducing and stabilizing agent. The extract was mixed with a metal precursor solution (specific details such as metal salt type and concentration should be disclosed in future studies for reproducibility) under controlled temperature and pH conditions to facilitate nanoparticle formation. The reaction mixture was stirred continuously, and the formation of nanoparticles was confirmed by observing a color change, followed by characterization using SEM and DLS. Additionally, the sample size for the study was determined based on prior research and statistical power calculations, ensuring that the number of animals per group was sufficient to detect significant differences with a confidence level of 95% and a power of 80%. These refinements strengthen the methodological transparency and enhance the reproducibility of the study.

RESULTS

The study assessed the anti-cancer efficacy of *Aloe vera* nanoparticles (AVNPs) in oral cancer-induced rat tissues, focusing on cytotoxicity and pro-apoptotic activities. Tissue-derived cells were exposed to serial dilutions of AVNPs to determine their toxicity using the *MTT* assay. The 24-hour assay was selected due to its high reproducibility and consistent results, yielding an *IC50* value of 13.7 μM for AVNP treatment. This was compared to the *IC50* of Cisplatin, which served as the control treatment. The findings demonstrated that AVNPs exhibited comparable cytotoxic effects against tumor cells, highlighting their potential as an effective therapeutic agent.

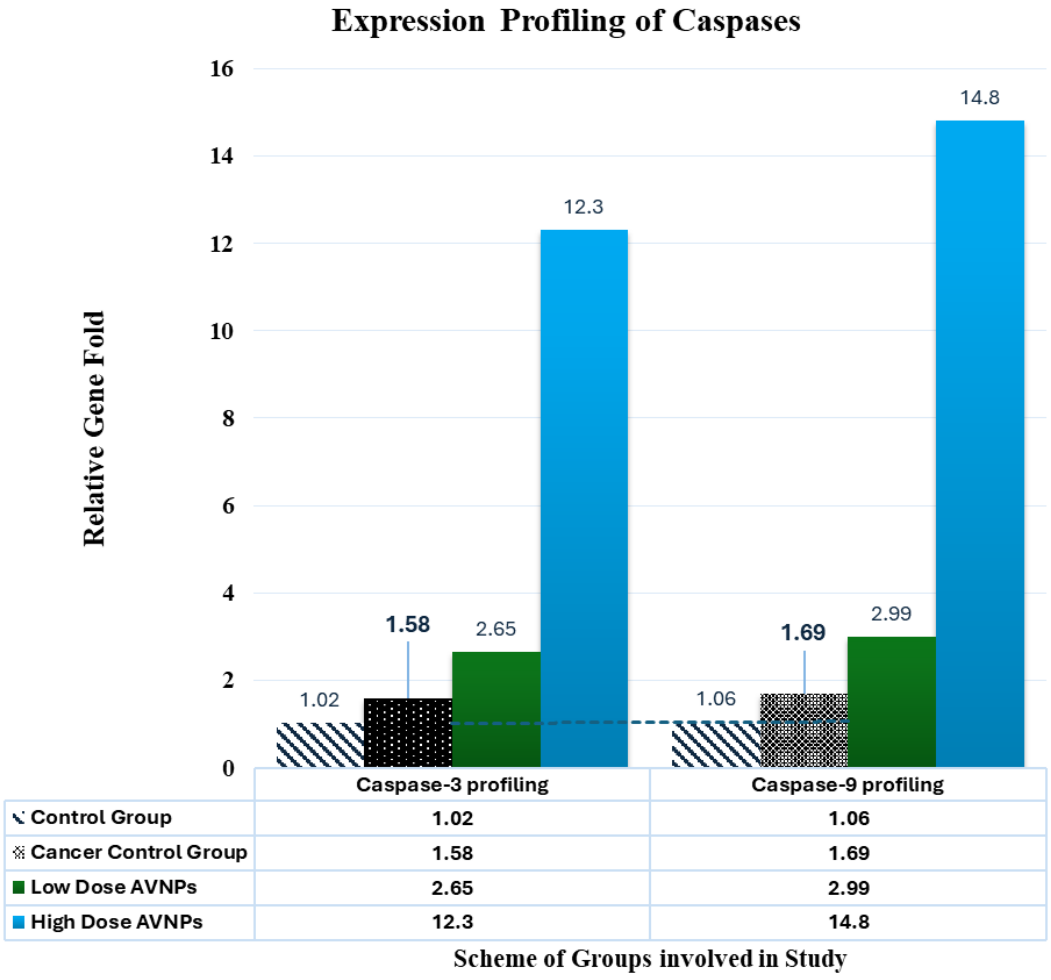

Figure 1. Comparative analysis of *Caspase* levels

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Gene expression analysis revealed significant pro-apoptotic activity in treated tissues. The relative fold expression of *Caspase-3* and *Caspase-9* genes, which are key markers of apoptosis, was notably elevated in tissues treated with AVNPs compared to untreated controls. *Caspase-9* expression reached a relative fold increase of 14.8, which was significantly higher than the 12.3 observed for *Caspase-3*. These results confirm the potent pro-apoptotic and tumor-suppressive effects of AVNPs. The findings emphasize the therapeutic potential of AVNPs, particularly their ability to induce programmed cell death and inhibit tumor growth. These outcomes demonstrate that AVNPs not only possess significant cytotoxic effects on tumor cells but also effectively upregulate apoptotic pathways, providing evidence of their utility in oral cancer treatment. However, additional results, such as histopathological changes, tumor size reduction, or broader molecular analyses, could further substantiate the findings and address the study's objectives comprehensively.

DISCUSSION

Aloe vera has long been recognized for its medicinal properties, including antioxidative, anti-inflammatory, pro-apoptotic, anti-proliferative, and anti-tumorigenic effects, making it a promising candidate for cancer therapeutics (12). The findings of this study further substantiate the anticancer potential of *Aloe vera* nanoparticles (AVNPs), particularly in oral cancer models. The increased expression of apoptosis-related genes, such as *Caspase-3* and *Caspase-9*, strongly supports the hypothesis that AVNPs exert their effects by inducing intrinsic apoptosis. This mechanism effectively inhibits cancer cell proliferation, thereby impeding tumor growth. These observations align with prior studies demonstrating the ability of *Aloe vera* and its derivatives to activate pro-apoptotic pathways and disrupt tumor progression (13, 14). The study's results also mirror findings from investigations of other natural compounds, such as piperine and cinnamaldehyde, which have shown similar effects in activating apoptosis and altering cancer cell integrity (15, 19). For example, in breast cancer models, piperine induced apoptosis via *Caspase* activation, while cinnamaldehyde disrupted the structural integrity of leukemia cells, causing organelle fragmentation and membrane rupture (16, 20). Similarly, *Aloe vera* has demonstrated the ability to inhibit proliferation and metastasis at concentrations as low as 8 μM in breast cancer cells by impacting molecular markers such as matrix metalloproteinases (MMPs), further solidifying its broad-spectrum anticancer efficacy (17). In the context of oral cancer, these results suggest that AVNPs not only offer targeted tumor suppression but also preserve healthy tissues, a significant advantage over conventional therapies (24).

The ability of *Aloe vera* to induce apoptosis has been confirmed across various cancer types, including liver (HepG2) and breast cancer models, with studies utilizing techniques such as flow cytometry and fluorescence microscopy. Apoptotic nuclear fragmentation visualized using *Hoechst 33258* dye underscores the precision with which *Aloe vera* induces programmed cell death, further validating its therapeutic role (23). These cellular changes are attributed to the nanoparticle-mediated enhancement of membrane permeability, resulting in cellular disintegration and the subsequent inhibition of tumor growth (21, 22). The findings of this study highlight the therapeutic promise of AVNPs in the management of oral cancer. However, certain limitations warrant consideration. While the study demonstrated the efficacy of AVNPs in inducing apoptosis and inhibiting tumor growth, additional molecular analyses are needed to further elucidate the underlying mechanisms. Techniques such as *High-Performance Liquid Chromatography (HPLC)* could aid in isolating and identifying specific phytochemicals within *Aloe vera* that contribute to its anticancer activity, enhancing specificity and potency (25). Moreover, the interactions of AVNPs with other anti-proliferative and anti-metastatic biomarkers remain an area of unexplored potential.

Future studies should focus on the translation of these findings to clinical applications. Comprehensive *in vivo* investigations incorporating detailed histopathological assessments, tumor size reduction data, and broader molecular profiling could strengthen the evidence base. Additionally, the role of *Aloe vera* as a natural adjuvant in oncology holds significant potential, particularly in reducing the systemic toxicity associated with conventional therapies. Incorporating clinical trials would provide critical insights into its efficacy and safety in human populations, paving the way for its integration into personalized cancer management strategies. While this study demonstrates the strengths of AVNPs in providing a natural, targeted therapeutic option for oral cancer, it also highlights the need for further research to optimize its clinical applicability. Addressing these gaps could enhance the understanding of its molecular mechanisms and position *Aloe vera* nanoparticles as a viable tool in the advancement of cancer therapeutics.

CONCLUSION

This study reaffirms the pro-apoptotic potential of *Aloe vera* in targeting oral cancer cells, highlighting its ability to disrupt cancer cell metabolism and induce programmed cell death. *Aloe vera* nanoparticles offer a promising chemoprotective approach with the potential to enhance the effectiveness of cancer therapies while minimizing systemic toxicity. These findings emphasize the need for further research and clinical trials to explore the therapeutic efficacy of *Aloe vera*, particularly in its nanoparticle form, as a targeted treatment option for oral cancer and other malignancies.

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AUTHOR CONTRIBUTIONS

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Sawera Saleem Khan	Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Validation, Supervision
Muhammad Arslan Zaffar	Methodology, Investigation, Data Curation, Writing - Review & Editing
Soobia Pathan	Investigation, Data Curation, Formal Analysis, Software
Muhammad Sheryar	Software, Validation, Writing - Original Draft
Muhammad Akram	Formal Analysis, Writing - Review & Editing
Muhammad Arsalan	Writing - Review & Editing, Assistance with Data Curation

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