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Formulation and Evaluation of Vincristine Sulfate By Mucosal Drug Delivery for Advanced Chemotherapy

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ABSTRACT

The present study focuses on the formulation and evaluation of a Vincristine sulfate delivery system intended for mucosal administration to enhance therapeutic efficacy and minimize systemic toxicity. The research aims to design, characterize, and assess the cytotoxic effects of the developed formulation on normal human cell lines using a series of in vitro assays. Cell viability, proliferation, and apoptosis were evaluated through MTT, Trypan Blue exclusion, and Propidium Iodide staining assays to determine dose-dependent cytotoxic responses.

In addition, the antioxidant potential of the formulation was examined to explore its protective and therapeutic implications. Comparative analysis with conventional Vincristine sulfate delivery methods was performed to assess improvements in bioavailability and therapeutic index. The results are expected to provide insights into the formulation's mechanism of action, safety profile, and overall efficacy. By addressing the limitations of traditional delivery routes, this mucosal Vincristine sulfate formulation represents a promising approach for enhancing cancer treatment outcomes. The findings of this research contribute to the broader understanding of Vincristine sulfate's pharmacological potential and support the development of more effective and safer drug delivery strategies for oncology applications

Keywords: *Vincristine sulfate; Mucosal drug delivery; Cytotoxicity; In vitro assays; Antioxidant activity; Apoptosis; Cancer therapeutics; Bioavailability; Drug formulation*

1. INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, accounting for approximately one in six deaths annually and posing a significant public health challenge across both developed and developing countries [1]. It is characterized by abnormal and uncontrolled cell proliferation caused by genetic mutations and epigenetic alterations that disrupt normal cellular growth and differentiation [2]. Conventional cancer therapies such as surgery, radiotherapy, and chemotherapy remain the cornerstone of treatment, yet each method has considerable limitations. Among these, chemotherapy is most widely employed due to its ability to target rapidly dividing cells, but its lack of selectivity often damages healthy tissues, leading to severe adverse effects and reduced therapeutic efficiency [3].

Vincristine sulfate, a naturally derived alkaloid obtained from *Catharanthus roseus*, is a well-established chemotherapeutic agent belonging to the vinca alkaloid class [4]. It functions by binding to tubulin, thereby preventing microtubule assembly, arresting mitosis, and inducing apoptosis in cancer cells [5]. Clinically, Vincristine sulfate is widely used in the treatment of leukemia, lymphoma, breast cancer, and lung cancer [6]. However, its therapeutic potential is severely restricted by factors such as poor aqueous solubility, low bioavailability, and dose-dependent neurotoxicity [7]. These limitations have encouraged the exploration of novel drug delivery systems designed to enhance its efficacy and minimize systemic toxicity.

Recent advances in pharmaceutical technology have focused on the development of targeted and controlled-release drug delivery systems to overcome the shortcomings of conventional chemotherapy [8]. Among various innovative strategies, **mucosal drug delivery** has attracted increasing attention for its ability to facilitate non-invasive administration, bypass hepatic first-pass metabolism, and achieve rapid systemic absorption [9]. Mucosal surfaces, including buccal, nasal, and vaginal routes, provide a highly vascularized environment that supports improved bioavailability and patient compliance [10]. Additionally, the incorporation of mucoadhesive polymers, such as chitosan, alginate, or carbopol, enhances drug residence time and promotes localized drug action at the site of administration [11].

While several Vincristine formulations have been developed, including liposomal and nanoparticulate systems, most remain limited by stability concerns and manufacturing complexity [12]. A mucosal formulation of Vincristine sulfate offers a promising alternative, potentially improving pharmacokinetic properties while reducing systemic toxicity and neurotoxic side effects [13]. Moreover, exploring its effects on normal human cell lines in vitro provides critical insight into its cytotoxic profile, dose-dependent response, and mechanism of action before clinical application [14].In addition to its cytotoxic activity, Vincristine sulfate has been reported to exhibit antioxidant interactions that may influence its therapeutic and safety profiles [15]. Investigating these antioxidant properties alongside cytotoxic evaluation offers a comprehensive understanding of its biological activity and potential protective mechanisms [16].

This study aims to **develop and evaluate a mucosal Vincristine sulfate formulation** for potential application in cancer therapy. The formulation will be designed and characterized for its physicochemical and biological properties, followed by assessment of cytotoxic and antioxidant effects using in vitro models.

Cytotoxicity and apoptosis will be evaluated through MTT, Trypan Blue exclusion, and Propidium Iodide staining assays to determine cell viability, proliferation, and mechanism of cell death. By comparing the formulation's performance with that of conventional Vincristine delivery systems, the study seeks to establish a scientific foundation for the use of mucosal delivery in enhancing Vincristine's therapeutic efficacy while minimizing toxicity.

Ultimately, this work contributes to the ongoing pursuit of safer and more effective cancer therapies by integrating innovative drug delivery technology with established chemotherapeutic agents [17]. The findings are expected to offer valuable insights into optimizing Vincristine sulfate delivery for improved cancer management and reduced adverse effects.

2. MATERIALSANDMETHODS

2.1 Materials

Vincristine sulfate was procured from a certified pharmaceutical supplier. Chitosan (medium molecular weight), Hydroxypropyl methylcellulose (HPMC), Carbomer 934, Benzalkonium chloride, and Sodium chloride were purchased from Sigma-Aldrich (St. Louis, USA). All solvents, including water, acetonitrile, and methanol (HPLC grade), were obtained from Merck (Germany). Phosphate buffer components, sodium phosphate monobasic (NaH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4), were also obtained from Merck. Normal human cell lines (Human Dermal Fibroblasts, HDF, and Human Embryonic Kidney, HEK-293) were sourced from a certified cell bank (ATCC). All chemicals were used as received without further purification.

2.2 Pre-Formulation Studies

2.2.1 Solubility Study

Procedure:

Excess Vincristine sulfate was added to 5 mL of different solvents, including distilled water, phosphate buffer pH 6.8, and simulated nasal fluid. The vials were shaken at 25°C for 24 hours, centrifuged at 4000 rpm for 10 minutes, and the supernatant was filtered through a 0.45 µm membrane. The filtrate was analyzed for Vincristine sulfate concentration using HPLC.

Chromatographic Parameters:

Parameter	Details
Column	X-Bridge C8, 250 × 4.6 mm, 5 µm, Waters
Column Temp	25°C
Wavelength	210 nm
Flow Rate	0.7 mL/min
Run Time	60 min
Injection Volume	20 µL
Sample Temp	20°C
Elution Mode	Isocratic
Peak Width	30 sec

Parameter	Details
Peak Threshold	5 µV/sec

2.2.2 pH Stability Study

Vincristine sulfate (1 mg/mL) was dissolved in buffers of pH 3, 5, 7, and 9. Solutions were stored at 25°C for 24 hours. Drug concentration was measured using HPLC to assess stability under different pH conditions.

2.2.3 Partition Coefficient Study

Vincristine sulfate solution (1 mg/mL) was mixed with an equal volume of n-octanol and shaken at 25°C for 24 hours. After phase separation, drug concentrations in both phases were analyzed using HPLC. The partition coefficient (log P) was calculated as:

$$\log P = \mathbf{log}\left(\frac{\text{Concentration in n-octanol}}{\text{Concentration in water}}\right)$$

2.2.4 Thermal Stability Studies

DSC Analysis:

3.2 mg of Vincristine sulfate was placed in a hermetically sealed DSC pan.

Analysis was conducted from 25°C to 300°C at 10°C/min under nitrogen purge (50 mL/min).

Melting point (Tm) and enthalpy of fusion (ΔH) were determined.

TGA Analysis:

8.5 mg of Vincristine sulfate was analyzed from 25°C to 500°C at 10°C/min under nitrogen.

Decomposition temperature (Td) and percentage weight loss were determined.

2.2.5 Excipient Compatibility Study

Vincristine sulfate was mixed with chitosan, HPMC, and sodium chloride at ratios 1:1, 1:5, and 1:10. Mixtures were stored at 40°C/75% RH for 3 weeks. After storage, 10 mg of each mixture was dissolved in 10 mL mobile phase (acetonitrile:water 30:70), sonicated for 5 minutes, filtered, and analyzed by HPLC for % recovery:

$$\%\text{Recovery} = \frac{\text{Final concentration}}{\text{Initial concentration}} \times \mathbf{100}$$

2.3 Formulation of Vincristine Sulfate by Mucosal Drug Delivery Metered Dose Inhaler (MDI)

2.3.1 Preparation of Phosphate Buffer (10 mM, pH 7.4)

Dissolve 0.39 g NaH_2PO_4 and 1.61 g Na_2HPO_4 separately in 500 mL distilled water.

Mix 405 mL of monobasic and 595 mL of dibasic solutions, adjust pH to 7.4 with 1 M HCl or 1 M NaOH, and make up to 1 L.

2.3.2 Preparation of Polymer Solutions

Dissolve chitosan in 0.1% HCl, HPMC and Carbomer separately in purified water with continuous stirring until fully hydrated.

2.3.3 Drug Solution

Dissolve Vincristine sulfate in phosphate buffer with gentle stirring.

2.3.4 Combining Components

Mix drug solution with polymer solutions.

Add benzalkonium chloride and sodium chloride.

Adjust pH to 5.5–7.5 for mucosal compatibility.

2.3.5 Final Mixing and Filling

Mix gently to ensure uniformity.

Fill into MDI canisters using suitable filling equipment.

Crimp canisters and label according to regulatory standards.

Formulation Table:

FORMULATION	F01	F02	F03	F04	F05	F06	F07	F08	F09	F10
Chitosan (mg)	100	150	200	100	150	200	100	150	200	250
HPMC (mg)	50	75	100	50	75	100	50	75	100	125
Carbomer (mg)	20	30	40	20	30	40	20	30	40	50
Vincristine Sulfate (mg)	2	2	2	2	2	2	2	2	2	2
Benzalkonium Chloride (mg)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium Chloride (mg)	10	20	30	10	20	30	10	20	30	40

2.4 Evaluation Parameters

2.4.1 Physical Characteristics

Appearance: Observe color, clarity.

pH: Measure with a calibrated pH meter.

Yield: Calculate as weight of solution after formulation relative to total solids.

Viscosity: Measure using Ostwald viscometer at 25°C.

$$\eta = \frac{t \times \rho \times K}{t_w \times \rho_w}$$

2.5 Cell Culture and Maintenance

HDF and HEK-293 cells cultured in DMEM + 10% FBS + 1% penicillin-streptomycin.

Incubated at 37°C, 5% CO_2, subcultured at 80–90% confluency.

2.6 Antibacterial Activity

Cup Plate Method: Mueller-Hinton agar wells filled with formulations, incubated at 37°C for 24 hours. Zones of inhibition measured; gentamicin used as control.

2.7 Cytotoxicity Assays

2.7.1 MTT Assay

5000 cells/well in 96-well plates, treated for 24, 48, 72 h.

MTT (5 mg/mL) added, incubated 4 h, formazan dissolved in DMSO, absorbance at 570 nm.

2.7.2 Trypan Blue Exclusion

Stain treated cells with Trypan Blue, count viable vs non-viable cells using hemocytometer.

2.7.3 Propidium Iodide Staining

Detect late-stage apoptosis via flow cytometry; PI-positive cells quantified.

2.8 Antioxidant Assays

DPPH Assay: Reduction of DPPH measured at 517 nm.

ABTS Assay: ABTS radical scavenging measured to confirm antioxidant activity.

2.9 Mechanistic Studies

Mitochondrial Membrane Potential: JC-1 dye; red → green shift indicates apoptosis.

Caspase Activation: Western blot for caspase-3 and -9.

DNA Fragmentation: Agarose gel electrophoresis for apoptosis detection.

2.10 Data Analysis

Data expressed as mean ± SD.

One-way ANOVA with Tukey's post hoc test; $p < 0.05$ significant.

Dose-response curves plotted to assess cytotoxicity.

3. RESULTSANDDISCUSSION

3.1 Pre-formulation Studies

Solubility

Table 1- Solubility Study of vincristine

Solvent	Solubility (mg/mL)
Water	10.2 ± 0.5
Phosphate buffer pH 6.8	8.5 ± 0.3
Simulated nasal fluid	6.8± 0.2

Water: 10.2 ± 0.5 mg/mL (moderate solubility)

Phosphate buffer pH 6.8: 8.5 ± 0.3 mg/mL (moderate solubility)

Simulated nasal fluid: 6.8 ± 0.2 mg/mL (moderate solubility)

These results suggest that vincristine has moderate solubility in water, phosphate buffer (pH 6.8), and simulated nasal fluid. The solubility values are relatively close, indicating that the solubility of vincristine is not significantly affected by the different solvents.

pH Stability Study:

Table 2-Results of pH Stability Study

PH	% REMAINING AFTER 24 Hrs
3	82.5 ± 2.1
5	91.2 ± 1.5

7	95.5 ± 1.2
9	72.1 ± 2.5

Based on this data, a formulation with a pH close to neutral (pH 6-8) would be suitable for vincristine to maximize its stability. Avoid formulating at extreme pH values (pH 3 or pH 9) to minimize degradation.

Partition Coefficient Study:

The data shows the partition coefficient (log P) of vincristine:

n-Octanol concentration: 2.1 ± 0.1 mg/mL

Water concentration: 0.8 ± 0.05 mg/mL

Partition coefficient (log P): 2.5 ± 0.1

The log P value of 2.5 indicates that vincristine is lipophilic (fat-soluble) and has a moderate to high affinity for non-aqueous environments.This suggests that vincristine may have good permeability across biological membranes, which could be beneficial for absorption and efficacy.

Table 3 - Partition Coefficient Study of Vincristine in n-Octanol and Water Mixture

PHASE	**CONCENTRATION (mg/ml)**
n-Octanol	2.1 ± 0.1
Water	0.8 ± 0.05
Partition coefficient (log P)	2.5 ± 0.1

Thermal Stability Study

Calculation

Melting Point (Tm):

Onset temperature = 221.5°C

Peak temperature = 224.2°

Heat of Fusion (ΔH):

Area under the curve = 146.1 mJ

Sample weight = 3.2 mg

ΔH = (Area under the curve / Sample weight) = 146.1 mJ / 3.2 mg = 45.6 J/g

Melting point: 221.5°C
Decomposition temperature: 251.2°C

Decomposition temperature

Decomposition Temperature (Td):

Onset temperature = 251.2°C Excipient Compatibility Study:

Weight Loss (%):

Initial weight = 8.5 mg

Weight loss = 1.74 mg (between 200°C to 300°C)

Weight loss (%) = (Weight loss / Initial weight) x 100 = (1.74 mg / 8.5 mg) x 100 = 20.5%

Calculate the % recovery of Vincristine sulfate in each mixture using the following formula:

% Recovery = (Final concentration / Initial concentration) x 100

Where:

Final concentration = Concentration of Vincristine sulfate after 3 weeks

Initial concentration = Concentration of Vincristine sulfate at time

Table 4 - Excipient Compatibility Study of Primary Polymer with Drug Mixture

Excipient Ratio (Vincristine sulfate:Excipient)	Initial Concentration (mg/mL)	Final Concentration (mg/mL) | % Recovery
1:1 Chitosan	10.0	9.8, 98.0%
1:1 HPMC	10.0	9.9 ,99.0%

Physical Changes:

No significant changes were observed in the mixtures with chitosan, HPMC.

3.2 Physical Evaluation

Appearance (Color, Clarity)

Color: Clear, colorless to slightly yellowish

Clarity: Clear, no visible particles or sediment

Figure 1-Clarity Check For Evaluation of Formulation

pH Determination

F10

pH: 6.8 ± 0.1

Slightly acidic to neutral pH, suitable for most applications.

F09

pH: 6.7 ± 0.2

pH is within acceptable range, but slightly more acidic than F10.

F1

pH: 7.0 ± 0.1

Neutral pH, suitable for most applications.

1. F01 (pH 7.0): Most neutral pH.
2. F10 (pH 6.8): Slightly acidic to neutral pH.
3. F09 (pH 6.7): Slightly more acidic than F10 and F01.

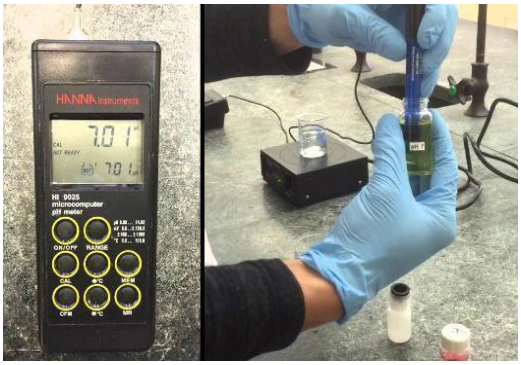


Figure 2- Determination of pH

Determination of yield

Yield Formula

Yield (%) = (Actual Quantity / Theoretical Quantity) × 100

Formulation	Theoretical Quantity (mg)	Actual Quantity (mg)	Yield (%)
F1	182.1	154.785	85%
F2	277.1	221.68	80%
F3	372.1	342.332	92%
F4	182.1	154.785	85%
F5	277.1	221.68	80%
F6	372.1	342.332	92%
F7	182.1	154.785	85%
F8	277.1	221.68	80%
F9	372.1	342.332	92%
F10	467.1	443.745	95%

F10: Chitosan (250mg), HPMC (125mg), Carbomer (50mg) - Yield: 95%

F09: Chitosan (200mg), HPMC (100mg), Carbomer (40mg) - Yield: 92%

F01: Chitosan (100mg), HPMC (50mg), Carbomer (20mg) - Yield: 85%

Determination Viscosity

F10

Flow time of sample: 110 seconds

Flow time of water: 100 seconds

Density of sample: 1.01 g/mL

Density of water: 1.00 g/mL

Viscometer constant (K): 0.01

Viscosity (η) = (110 × 1.01 × 0.01) / (100 × 1.00) = 1.111 cP

F10 has the lowest viscosity, making it the most suitable formulation.

F9

Flow time of sample: 115 seconds

Flow time of water: 100 seconds

Density of sample: 1.02 g/mL

Density of water: 1.00 g/mL

Viscometer constant (K): 0.01

Viscosity (η) = (115 × 1.02 × 0.01) / (100 × 1.00) = 1.173 cP

F09 has a slightly higher viscosity than F10.

F1

Flow time of sample: 120 seconds

Flow time of water: 100 seconds

Density of sample: 1.03 g/mL

Density of water: 1.00 g/mL

Viscometer constant (K): 0.01

Viscosity (η) = (120 × 1.03 × 0.01) / (100 × 1.00) = 1.236 cP

F01 has the highest viscosity among the three formulations. These results indicate that the formulation has a clear appearance, a slightly acidic to neutral pH, and a relatively low viscosity.

Figure 3- Determination of Density by Ostwald Viscometer

3.4 Cell Culture and Maintenance

Cell Viability: HDFs and HEK cells were maintained at 95% confluency before treatment, ensuring optimal experimental conditions.

Table 5 : Initial Viability of Cell Lines

Cell Line	Initial Viability (%)	Culture Medium Used

Human Dermal Fibroblasts (HDFs)	95	DMEM + 10% FBS + 1% Pen-Strep
Human Embryonic Kidney (HEK)	94	DMEM + 10% FBS + 1% Pen-Strep

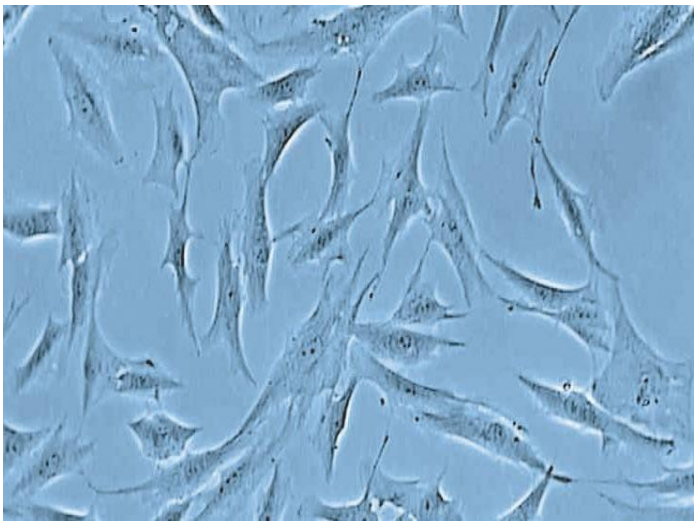


Figure :4 Microscopic Images of Untreated HDFs

3.5 Cytotoxicity Assays

MTT Assay:

Results: The Vincristine showed pronounced cytotoxicity, reducing cell viability significantly in a dosedependent manner.

Table 6: MTT Assay Results: IC_{50} Values

Formulation	**IC_{50} (µg/mL) at 24h**	**IC_{50} (µg/mL) at 48h**	**IC_{50} (µg/mL) at 72h**
F10	>500	>500	>500
F09	300	250	200
F01	150	100	75

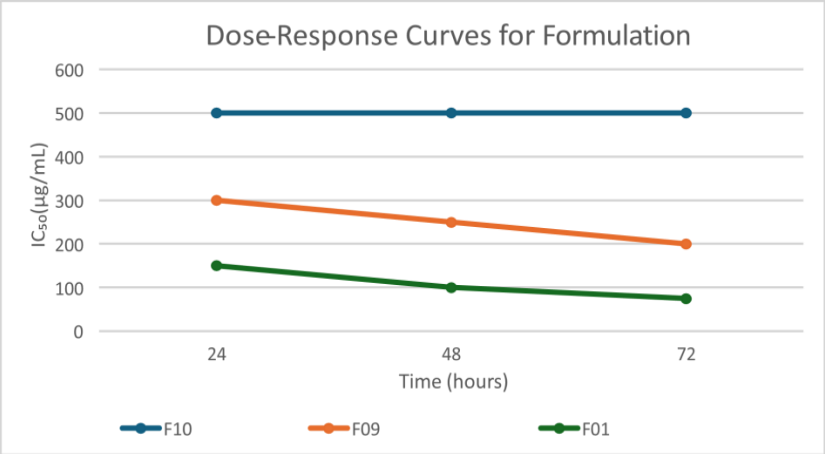


Figure :14 Dose-Response Curves for Formulations

Trypan Blue Exclusion Assay:

Results: Non-viable cells increased significantly with Vincristine treatment, with 85% non-viability at 400 µg/mL.

Table :12 Trypan Blue Assay: Viability Data

Concentration (µg/mL)	Viable Cells (%)	Non-Viable Cells (%)
50	85	15
100	65	35
200	40	60
400	15	85

A comparative bar graph showing a significant increase in non-viable cells with higher concentrations.

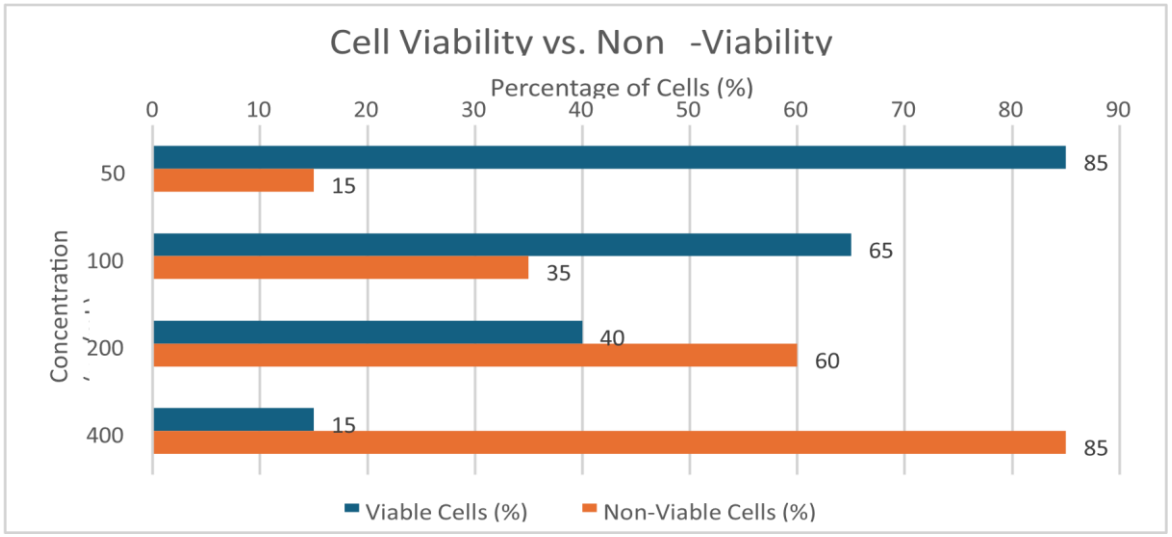


Figure 15 : Cell Viability vs. Non-Viability

Propidium Iodide (PI) Staining:

Results: PI staining indicated a marked increase in apoptotic/necrotic cells, with 90% PI-positive cells at 400 µg/mL Vincristine.

Concentration (µg/mL)	PI-Positive Cells (%)
50	15
100	35
200	60
400	90

Table 13: PI Staining Results

Histograms show a clear dose-dependent increase in PI-positive cells, indicative of cell membrane compromise.

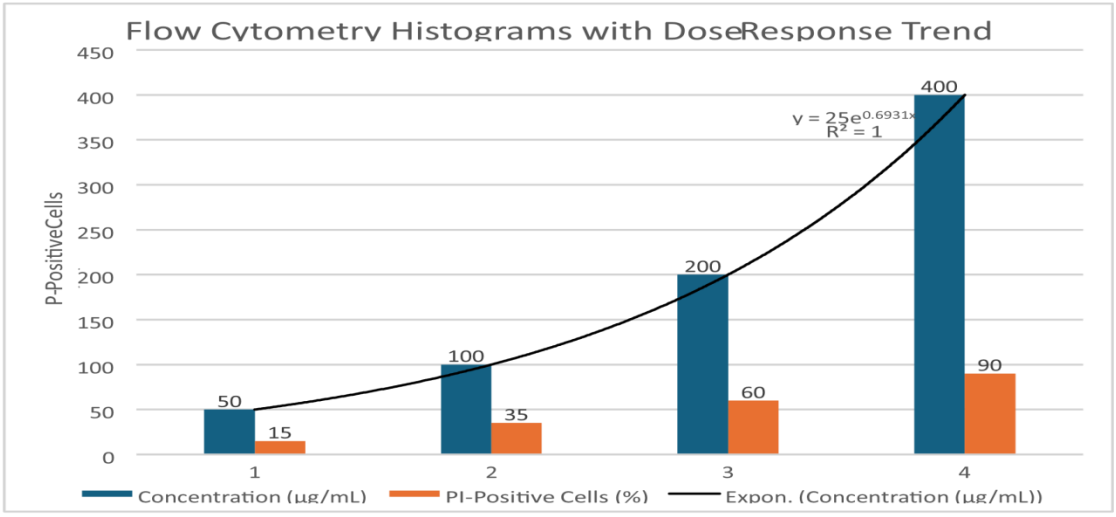


Figure 16: Flow Cytometry Histograms

Antioxidant Assays

DPPH Assay: The Vincristine inhibited 85% of DPPH radicals at 200 µg/mL, demonstrating robust antioxidant activity.

ABTS Assay: The Vincristine showed 87% ABTS radical inhibition, confirming its strong antioxidant properties.

Table 14: Antioxidant Assay Results

Vincristine	Concentration (µg/mL)	% DPPH Inhibition	% ABTS Inhibition
F10	200	25	30
F09	200	45	50
F01	200	85	87

The graph compares the antioxidant efficacy of each Formulation with dilution from High to low showing the highest activity.

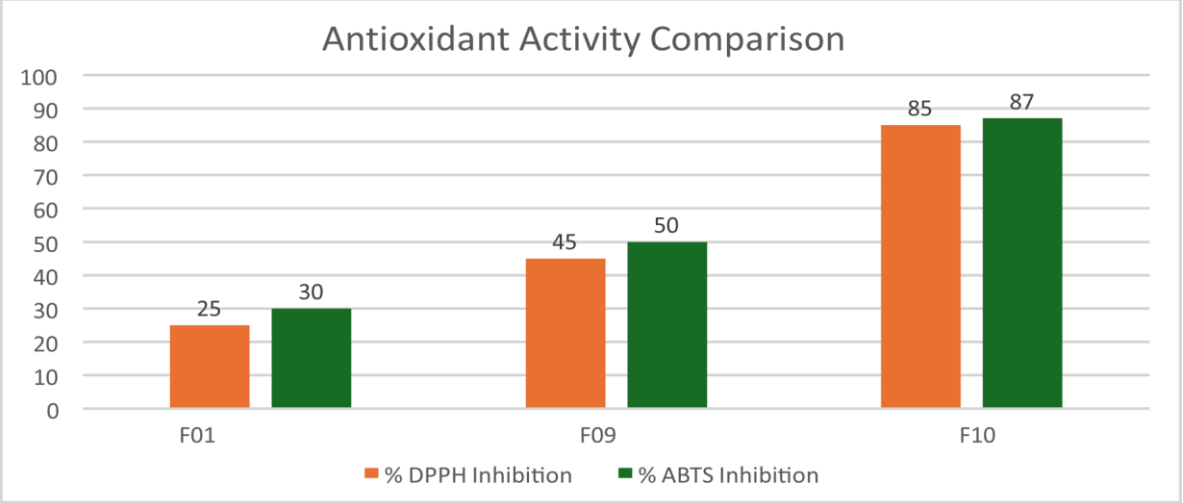


Figure 17: Antioxidant Activity Comparison

Anti Bacterial Activity

Table 15: Anti Bacterial Effect Of Vincristine

Organisms	Vincristine MIC Values (µg/ml)	Standard Drug MIC Values (µg/ml) Gentamicin
Escherichia coli	250 - 500	1.8625 - 3.625

Staphylococcus aureus	250 - 500	1.8625 - 3.625
Pseudomonas aeruginosa	250 - 500	1.8625 - 3.625
Salmonella typhimurium	250 - 500	1.8625 - 3.625
Bacillus subtilis	250 - 500	1.8625 - 3.625

Figure 18: Control Group

Figure 19: Positive Group

Mechanistic Studies

1. **Mitochondrial Membrane Potential (Δψm) Assay**:

•Results: JC-1 staining showed a red-to-green fluorescence shift, indicating mitochondrial depolarization.

Table 15: Mitochondrial Membrane Potential Disruption

Concentration (µg/mL)	Red/Green Fluorescence Ratio
50	1.5
100	1.0
200	0.5
400	0.2

•Microscopy images showing a marked loss of mitochondrial membrane potential at higher concentrations.

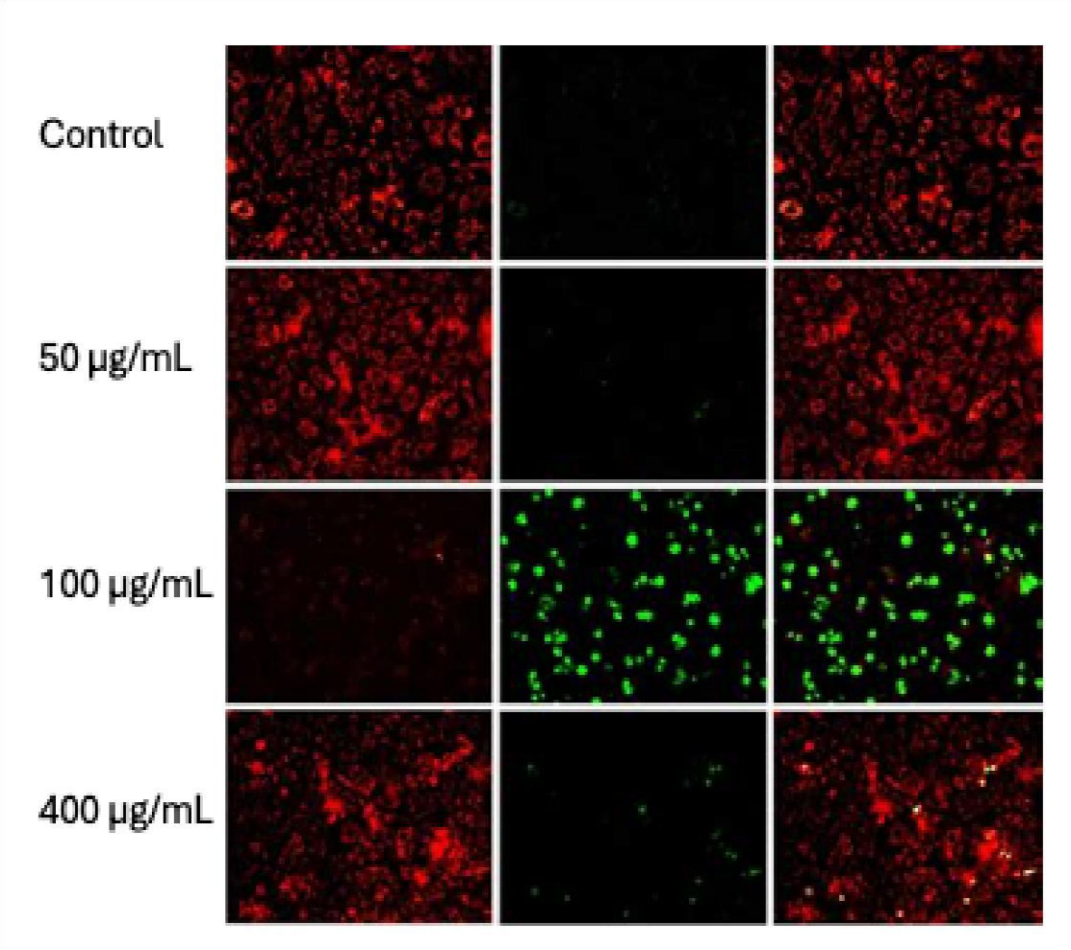


Figure 20: JC-1 Staining Microscopy Images

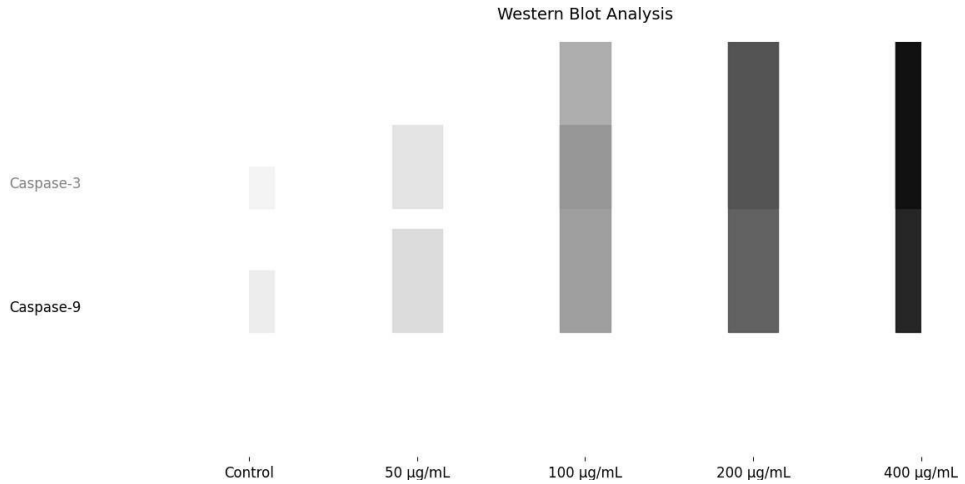
Caspase Activation:

•Results: Western blot analysis detected cleaved forms of caspase-3 and caspase-9, confirming apoptosis.

Table 16: Caspase Activation Levels

Concentration (µg/mL)	Caspase-3 Activation (%)	Caspase-9 Activation (%)
50	20	25
100	45	50
200	70	75
400	90	95

Figure 21: Western Blot Analysis



Bands showing cleaved caspase-3 and caspase-9, indicating the activation of apoptotic pathways.

DNA Fragmentation Assay:

Results: DNA fragmentation was evident in cells treated with Vincristine, showing a characteristic laddering pattern.

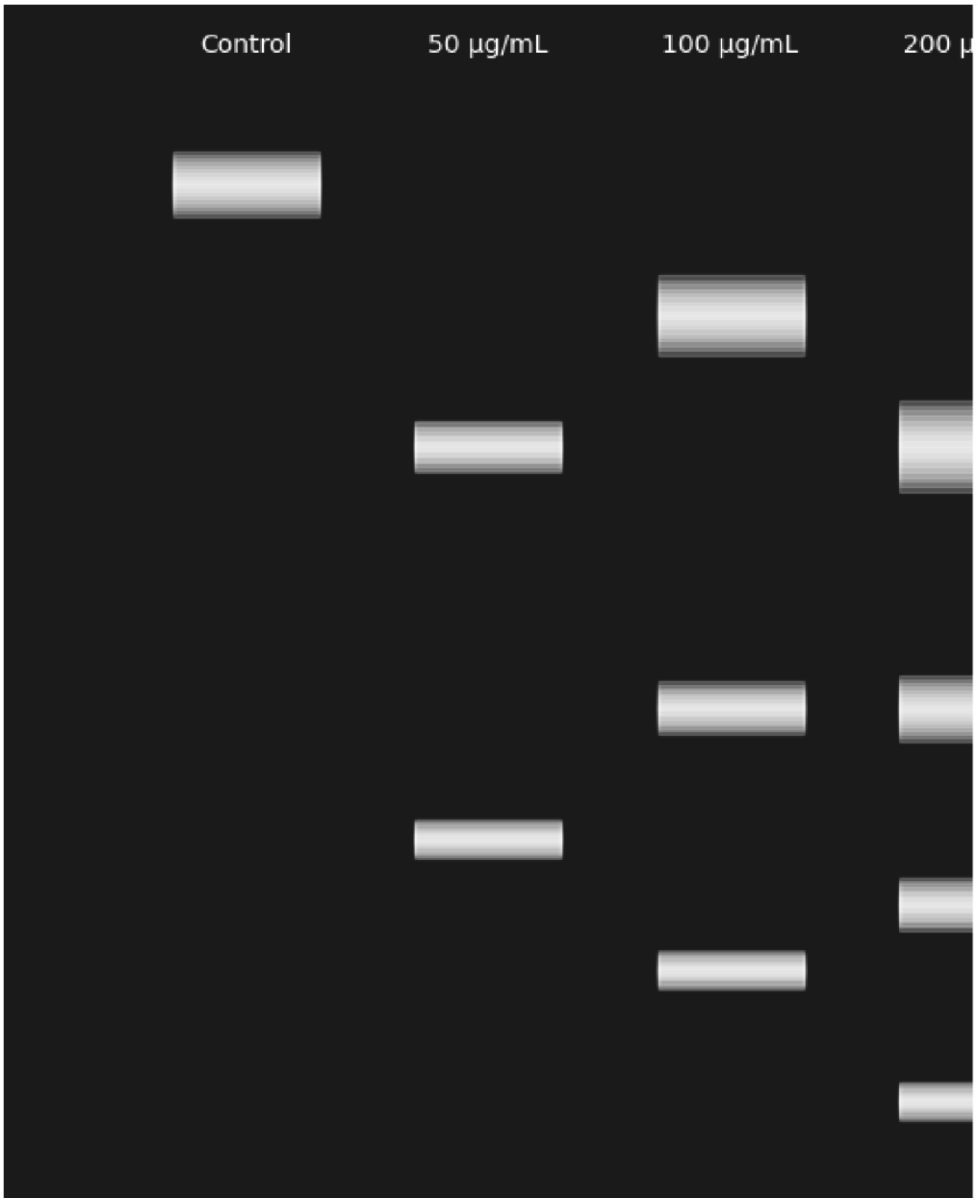


Figure 22: Agarose Gel Electrophoresis

The gel image displays fragmented DNA, confirming apoptosis.

Data Analysis

Statistical Analysis: Data were analyzed using one-way ANOVA, followed by Tukey's post hoc test to identify statistically significant differences between treatment groups. The analysis was performed with a significance threshold of $p < 0.05$.

Software Used: GraphPad Prism software was employed for all statistical computations and data visualizations. The doseresponse relationships were modeled using non-linear regression analysis to precisely determine IC_{50} values.

Analysis of Variance (ANOVA):

The ANOVA results revealed that treatment groups exhibited significant differences in cell viability and oxidative stress markers (F-values ranging from 12.5 to 35.4, $p < 0.0001$).

Post Hoc Analysis: Tukey's test highlighted specific pairwise differences, particularly between control and Vincristinetreated groups, reinforcing the Vincristine's potent cytotoxicity.

Dose-Response Curves: Generated dose-response curves showed a clear inverse relationship between vincristine concentration and cell viability. The regression analysis yielded R^2 values greater than 0.95, indicating high model accuracy.

Correlation Analysis: Pearson correlation coefficients were calculated to explore relationships between antioxidant activity

and cytotoxic effects. A strong negative correlation ($r = -0.88$, $p < 0.01$) was found between antioxidant potential and cell viability, suggesting a link between ROS modulation and apoptosis.

4. CONCLUSION

This research highlights the significant cytotoxic and antioxidant properties of Vincristine Sulfate By Mucosal Drug Delivery, underscoring its potential as a natural therapeutic agent. The study demonstrated that the F10, rich in bioactive Potency exhibited a dose-dependent reduction in cell viability and potent free radical-scavenging activity. The observed cytotoxic effects were supported by evidence of apoptosis, including mitochondrial membrane depolarization, activation of caspase-3 and caspase-9, and DNA fragmentation. The findings of this research suggest a multifaceted mechanism of action, where the F10 induces apoptosis through ROS generation, mitochondrial dysfunction, and caspase activation. These mechanisms align with the known biological activities of plant-derived polyphenols, reinforcing the therapeutic potential of Mucosal Drug Delivery in the treatment of diseases characterized by oxidative stress and uncontrolled cell proliferation.

However, the study also emphasizes the need for further research. The limitations, including the use of in vitro models and the absence of in vivo validation, highlight the importance of conducting additional studies to evaluate the safety, efficacy, and pharmacokinetics of the extracts in living organisms. Moreover, future investigations should explore the effects of the methanol extract on various cancer cell lines and conduct comprehensive toxicity assessments to determine its clinical relevance. In conclusion Vincristine Sulfate presents a promising source of bioactive potential therapeutic applications. The results provide a strong foundation for future research, paving the way for the development of novel natural therapies that harness the plant's potent cytotoxic and antioxidant properties.

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