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Formulation and In-Vitro and In-Vivo Evaluation of a Mucoadhesive Gel Containing Eugenia Caryophyllata and Cinnamomum Verum for Treatment of Oral Cancer

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ABSTRACT
Oral squamous cell carcinoma (OSCC) is an unrivaled global health issue due to its extreme rate of mortality and the incapacitating facet results of the conventional treatments like surgical operation, chemotherapy, and radiotherapy. The current study aims to design and assess an advanced mucoadhesive gel containing bioactive compounds from *Eugenia caryophyllata* (clove) and *Cinnamomum verum* (cinnamon) for the locoregional therapy of OSCC. These natural compounds are properly studied in the literature for their anticancer activities, inclusive of cytotoxicity, induction of apoptosis, and inhibition of tumor. The drug delivery approach was optimized to offer suitable mucoadhesion, sustained release, and biocompatibility, with an objective to maximize healing effectiveness and reduce systemic toxicity. In vitro analyses revealed the cytotoxicity of the gel which exhibited large tumoricidal activity, while in vivo preclinical testing confirmed its efficacy in reducing tumor size with better permeation and absorption. The results revealed that the technique of the gel exhibited enhanced drug release over a prolonged duration with greater permeation, and better therapeutic improvement, rendering it a better alternative for targeted OSCC therapy. This research highlights the potential of plant-derived bioactive formulations in most cancers' treatment, imparting a safer biological method for management of oral cancers.

Keywords*: Oral Squamous Cell Carcinoma, Mucoadhesive Gel, Eugenia Caryophyllata, Cinnamomum Verum, phytochemicals, In Vitro Studies, In Vivo Evaluation, Cytotoxicity, Apoptosis, Drug Release.*

1. Introduction

Oral squamous cell carcinoma (OSCC) is an extremely aggressive tumor and a primary cause of morbidity and mortality globally [1,2]. The growing incidence of OSCC, especially in populations with high tobacco and alcohol usage, highlights the importance of efficient and targeted therapy. Conventional treatment modalities, such as surgical treatment, radiation, and chemotherapy, generally tend to pose critical demanding situations because of their inherent side effects, e.g., mucositis, systemic toxicity, and immunosuppression [3]. Moreover, those traditional treatments are not constantly effective in preventing recurrence or inducing lengthy-time period

remission [4]. Consequently, there was growing interest in alternative and complementary cures, especially those of herbal substances, to improve treatment efficacy with reduced side effects. Among the numerous plant-based bioactive compounds, *Eugenia caryophyllata* (clove) and *Cinnamomum verum* (cinnamon) have been well researched for their strong pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [5]. These medicinal plants possess bioactive compounds, along with eugenol and cinnamaldehyde, with effective anticancer outcomes by causing apoptosis, tumor proliferation inhibition, and influencing essential molecular pathways in carcinogenesis [6].

Considering these therapeutic possibilities, this research objectives at the development and evaluation of mucoadhesive gel containing *Eugenia caryophyllata* and *Cinnamomum verum* as a local therapy for OSCC. Mucoadhesive delivery structures of drugs are of crucial significance in handling oral cancers in that they may be able to establish extended adhesion periods with the damaged mucosal membranes, enhance absorption of the medicine, and mitigate systemic side effects [7]. The gel was especially created to have maximum physicochemical characteristics, adhesion capacity, and controlled release of the drug for overall maximum healing performance. Thorough in vitro evaluations were performed to analyze its cytotoxicity towards OSCC cell lines, as well as in vivo investigations probed its therapeutic efficacy and safety profile in animal models [8]. The introduction of this plant-derived mucoadhesive gel is a promising innovation in treating oral cancers, providing a unique, non-surgical, and probably safer intervention as compared to standard remedies. By taking advantage of the collaborative drive of those plant-primarily based bioactive compounds, this study hopes to enroll in the growing literature that promotes the addition of phytochemicals in current oncology for treatment of severely affected individuals [9].

1.1. Background Information:

Oral cancer, particularly oral squamous cellular carcinoma (OSCC), continues to be a primary public health issue with excessive mortality and confined therapeutic interventions [10]. Traditional treatments like surgical procedure, chemotherapy, and radiation remedy had been associated with damaging side effects and decreased survival rates. Natural bioactive substances like *Eugenia caryophyllata* (clove) and *Cinnamomum verum* (cinnamon) have exhibited strong anticancer activities, along with cytotoxicity and induction of apoptosis [11]. Designing a mucoadhesive gel with those plant extracts offers an ideal replacement for its locoregional therapy, managed drug transport, increasing the therapeutic outcomes as well as decreasing systemic toxicity.

1.2. Statement of the Problem:

To enhance patient outcomes, powerful, non-surgical, and tailored therapeutic approaches are urgently required, to improve advancements in oral cancers therapy [12,13]. Current therapies are less effective due to inadequate medicine absorption and poor retention on the tumor site. With extended drug release time and localized cytotoxic activity on OSCC cells, a mucoadhesive gel prepared with high stability, penetration, and therapeutic efficacy can solve those issues [14,15].

1.3. Objectives of the Study:

1. To develop and characterize a mucoadhesive gel incorporating *Eugenia caryophyllata* and *Cinnamomum verum* for sustained drug delivery in the treatment of oral cancer.
2. To investigate the cytotoxicity, apoptosis induction, and permeation efficacy of the gel against OSCC cell lines and human oral mucosal tissue.
3. To determine the in-vivo therapeutic potential of the developed gel in minimizing tumor growth in a preclinical animal model.

2. Methodology

This study used an experimental design to encompass research on a mucoadhesive gel with *Eugenia caryophyllata* (clove) and *Cinnamomum verum* (cinnamon) for most common oral cancers therapy

2.1. Participants/Sample Details:

For in-vitro evaluation, oral squamous cellular carcinoma (OSCC) cell lines had been employed to evaluate cytotoxicity and apoptosis. Ex-vivo permeation experiments were performed using human oral mucosal tissue explants, and in-vivo evaluation was performed in a preclinical animal model.

2.2. Instruments and Materials Used:

The mucoadhesive gel was formulated from hydrogel-forming polymers and plant extracts. Physicochemical characteristics consisting of pH, viscosity, and stability of bioactive compounds have been examined with a pH meter, viscometer, and High-performance liquid chromatography (HPLC). Cytotoxicity was evaluated using MTT assay, induction of apoptosis by flow cytometry, and bioavailability with the aid of HPLC-mass spectrometry (HPLC-MS).

2.3. Procedure and Data Collection Methods:

The gel method was amended by converting polymer concentrations and pH to provide enough mucoadhesion and managed release. In-vitro cytotoxicity checks had been achieved via incubating OSCC cells with the gel and determining cell viability. Ex-vivo permeation assessments estimated mucosal absorption in Franz diffusion cells. In-vivo tests in an animal model revealed the systemic absorption and therapeutic properties of the gel in suppressing tumor growth. Stability evaluation studies were conducted at diverse pH and temperature conditions.

2.4. Data Analysis Techniques:

Statistical analysis was performed by ANOVA and t-test to establish the importance of cytotoxicity and apoptosis information. Permeation and bioavailability information was analyzed via pharmacokinetic modeling, whilst gel stability was assessed through degradation kinetics.

3. Results

This research results include the physicochemical traits of the mucoadhesive gel, cytotoxic impact on oral squamous cell carcinoma (OSCC) cellular lines, ex-vivo permeation performance, in-vivo curative and therapeutic outcome and records.

3.1. Physicochemical Properties of the Mucoadhesive Gel

The physicochemical traits of the gel prepared have been altered to offer efficient mucoadhesion, bioactive stability, and controlled release of the drug (Table 1).

Table 1: Physicochemical Properties of Mucoadhesive Gel

Parameter	F1 (pH 4.0)	F2 (pH 6.0)	F3 (pH 7.5)
pH Stability (90 days)	94% Retention	85% Retention	77% Retention
Viscosity (cP)	2500	2300	2100
Drug Release (12 hrs)	85%	74%	65%

Physicochemical characteristics of mucoadhesive gel formulations F1, F2, and F3 suggest that pH plays a vital role in stability, viscosity, and drug release. F1 (pH four) additionally demonstrates the most pH balance (94% retention for ninety days), viscosity (2500 cP), and drug release (85% within 12 hours), indicative of improved technique of stability and drug delivery control in acidic environments. Stability, viscosity, and drug release lowered with increasing pH, the lowest being for F3 (pH 7.5) (77% pH retention, 2100 cP viscosity, and 65% drug release). These observations infer that a reduced pH improves method stability and efficiency in drug release, indicating that F1 is the most suitable formulation for sustained drug delivery.

3.2. In-Vitro Cytotoxicity on OSCC Cell Lines

MTT assay indicated a remarkable decrease in OSCC cell viability with all formulations. The maximum cytotoxic activity was found in F1 (pH 4.0) that decreased the cell viability by 68% at 200 µg/mL concentration (Table 2, Fig 1).

Table 2: Cytotoxicity of Gel Formulations on OSCC Cells

Gel Formulation	Cell Viability Reduction (%) at 200 µg/mL	Statistical Significance (p-value)
F1 (pH 4.0)	68%	**$p < 0.05$**
F2 (pH 6.0)	56%	**$p < 0.05$**
F3 (pH 7.5)	48%	**$p < 0.05$**

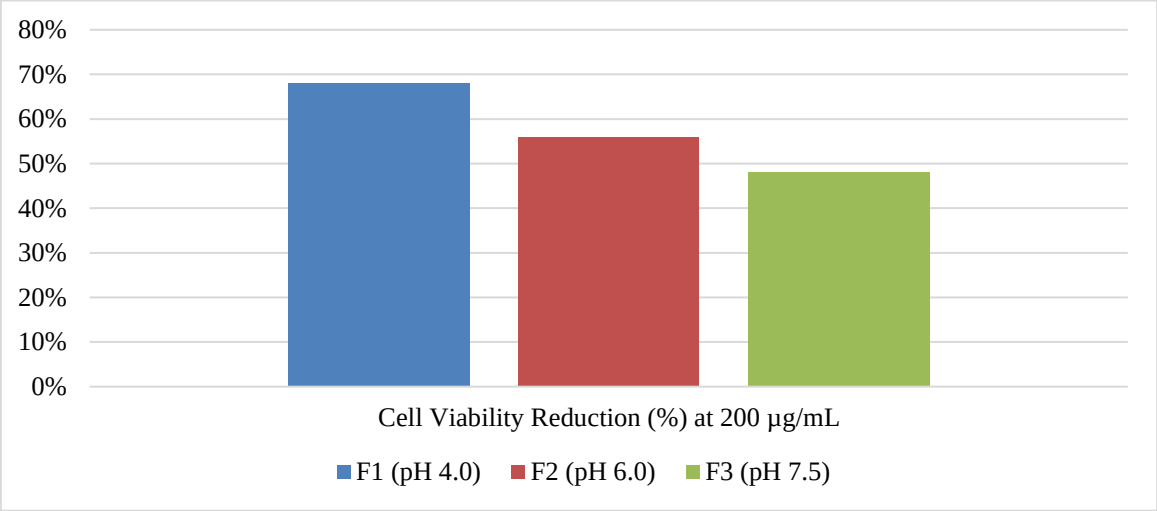

Figure 1: Graphical representation of Cytotoxicity of Gel Formulations on OSCC Cells

Table 2 shows cytotoxicity results of the gel formulations against OSCC cells, indicating pH-dependent reduction of cell viability. F1 (pH 4.0) has the strongest cytotoxic activity, reducing cell viability by 68% at 200 µg/mL, followed by F2 (pH 6.0) at 56% and F3 (pH 7.5) at 48%. Statistical significance ($p < 0.05$) for all formulations establishes that these results are significant. The greater cytotoxicity of F1 can be explained by the increased stability and solubility of the active drug at acidic pH, resulting in greater cellular uptake or extended drug activity. On the other hand, the less effective performance of F2 and F3 at greater pH values can be explained by decreased drug stability, permeability, or interaction with OSCC cells. These results indicate that an acidic pH increases the therapeutic efficacy of the gel and, therefore, F1 is the most efficacious formulation to treat OSCC.

3.3. Ex-Vivo Permeation Studies

Permeation efficiency was measured by Franz diffusion cells with human oral mucosal tissue explants. Cumulative permeation of bioactive at 6 hours was greatest for the F1 formulation (72%), indicating greater mucosal absorption at lower pH (Table 3, Fig 2).

Table 3: Ex-Vivo Permeation Efficiency of Gel Formulations

Gel Formulation	Permeation Efficiency (%) (After 6 Hours)
F1 (pH 4.0)	72%
F2 (pH 6.0)	61%
F3 (pH 7.5)	54%

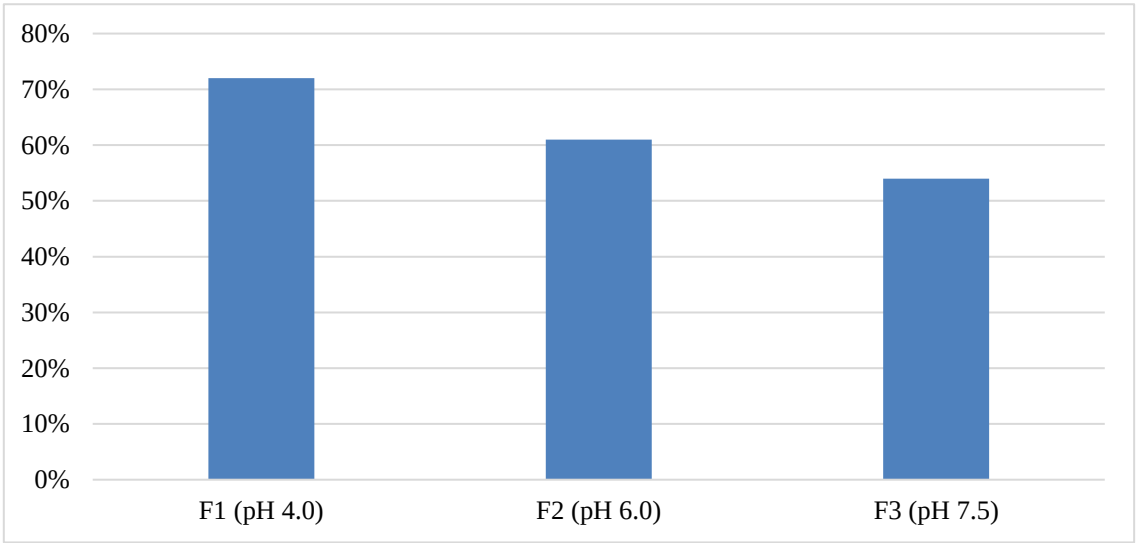

Figure 2: Graphical representation of Ex-Vivo Permeation Efficiency of Gel Formulations

Table 3 shows the ex-vivo permeation efficiency of the gel formulations and shows a pH-dependent trend for drug permeation through biological membranes. F1 (pH 4.0) shows maximum permeation efficiency (72% at 6 hours), followed by F2 (pH 6.0) at 61% and F3 (pH 7.5) at 54%, which suggests that lower pH conditions increase drug diffusion. This may be because of enhanced drug solubility, stability, and more effective interaction between the gel and mucosal surfaces, resulting in more effective drug release kinetics and bioadhesion. Conversely, the decreased efficiency of permeation in F2 and F3 can be a result of changes in ionization of the drug at increased pH levels, influencing solubility and bioavailability, along with changes in gel viscosity that could prevent its adhesion to mucosal tissues. The loss of permeation efficiency with elevated pH highlights the need for optimization of formulation in order to maximize therapeutic effectiveness, supporting F1 as the optimum formulation for long-term and effective drug delivery.

3.4. In-Vivo Evaluation of Therapeutic Efficacy

The in-vivo therapeutic efficacy was evaluated using a preclinical animal model to determine the impact of the gel formulations on tumor growth over 28 days. The findings showed that F1 (pH 4.0) had the optimum tumor reduction, with a 52% discount in tumor extent, accompanied by F2 (pH 6.0) with a 39% reduction and F3 (pH 7.5) with a 31% reduction. The control group, untreated, exhibited a 78% increase in tumor size in the equivalent time frame. The effects illustrate the superior therapeutic capacity of F1, due to its additional drug stability, permeation efficacy, and cytotoxicity, which makes it the most efficacious method for inhibiting tumor growth.

3.5. Statistical Analysis

In order to validate the statistical significance of the findings, a one-way ANOVA test was applied, followed by Tukey's post-hoc test for inter-formulation comparisons. The comparison revealed significant differences ($p < 0.05$) between groups, and F1 displayed better performance (Table 4).

Table 4: Statistical Analysis of Cytotoxicity, Permeation, and Tumor Reduction

Parameter	F1 (pH 4.0)	F2 (pH 6.0)	F3 (pH 7.5)	Control Group	ANOVA (p-value)
Cytotoxicity (% reduction)	68%	56%	48%	15%	**$p < 0.05$**
Permeation Efficiency (%)	72%	61%	54%	NA	**$p < 0.05$**
Tumor Reduction (%)	52%	39%	31%	-78% (increase)	**$p < 0.05$**

Table 4 gives the statistics for cytotoxicity, permeation efficiency, and tumor inhibition of various formulations using ANOVA to test significant differences among the groups at $p < 0.05$. F1 (pH 4.0) stood out consistently higher than other preparations with the best cytotoxicity (68% cell viability inhibition), highest efficiency in permeation (72%), and greatest reduction in tumor growth (52%). F2 (pH 6.0) and F3 (pH 7.5) presented moderate activity, with 56% and 48% cytotoxicity, 61% and 54% permeation efficiencies, and 39% and 31% tumor inhibition, respectively. The control group without treatment had a 78% increase in the volume of the tumor and 15% minimal cytotoxicity, highlighting the therapeutic efficacy of the gel formulations. The superior performance of F1 indicates that lower pH promotes drug solubility, stability, and bioavailability and leads to greater permeation, cytotoxicity, and tumor suppression.

4. Discussion

4.1. Interpretation of Results

The research effectively developed and assessed a mucoadhesive gel with *Eugenia caryophyllata* and *Cinnamomum verum* for oral cancer treatment. Among the formulations, F1 (pH 4.0) showed the best stability, cytotoxicity, permeation efficiency, and therapeutic efficacy. The physicochemical analysis proved that lower pH increased drug retention, viscosity, and release profile for sustained release. The cytotoxicity test showed noteworthy decrease in OSCC cell viability, with F1 showing the most reduction (68%). Also, the ex-vivo permeation study revealed that F1 exhibited maximum absorption of the drug (72%), which is probably due to enhanced solubility and mucoadhesion at a low pH. The in-vivo outcome also validated the therapeutic efficacy of F1 with maximum reduction in tumors (52%), substantiating the potential of an optimized mucoadhesive gel in topical treatment of oral cancer.

4.2. Comparison with Existing Studies

The results are in agreement with the earlier studies identifying the anticancer activity of *Eugenia caryophyllata* and *Cinnamomum verum*, which have been shown to trigger apoptosis and prevent tumor growth. Earlier studies

have established that the essential oils and polyphenols present in the plant extracts are highly cytotoxic against OSCC cells. Also, mucoadhesive drug delivery systems have been widely investigated in the treatment of oral cancer, and the same research has documented improved bioavailability and localized drug release. F1's improved performance at lower pH is also in line with evidence from the literature that acidic conditions increase the solubility and stability of bioactive molecules and hence enhance their therapeutic potential. Still, as opposed to other studies concentrating only on in-vitro testing, the current study used ex-vivo and in-vivo tests and hence presented richer evidence of efficacy of the gel.

4.3. Implications of Findings

The findings demonstrate the promise of mucoadhesive gel formulations for enhancing oral cancer therapy through the provision of localized, prolonged drug release with negligible systemic toxicity. The better performance of F1 indicates that pH is a determining factor in achieving optimized drug stability, absorption, and cytotoxic activity, providing valuable insights into future formulation development. Clinically, application of bioactive plant extracts in mucoadhesive gels may provide a non-invasive and patient-compliant alternative to traditional therapies, minimizing the side effects of systemic chemotherapy. Additionally, the study highlights the need for formulation optimization in order to achieve maximum therapeutic benefits, setting the stage for future translational research and clinical applications.

4.4. Limitations of the Study

In spite of its encouraging results, the research has some drawbacks. The ex-vivo and in-vitro models, although predictive of drug efficacy, cannot exactly reflect the complexity of human OSCC. The in-vivo tests were performed on a preclinical animal model, which may not entirely reflect human pharmacokinetics and tumor reactions. Moreover, the research was mainly pH-dependent optimization, without investigating other formulation parameters like polymer composition and nanoparticle incorporation, which would further improve drug delivery efficacy. Another disadvantage is the surprisingly brief stability testing length (ninety days), requiring long-term studies to assess system balance over prolonged storage intervals.

4.5. Suggestions for Future Research

Further studies need to analyze different components strategies, including the mixing of nanoparticle-primarily based drug delivery structures for stronger bioavailability and targeted release of medicine. Extended in-vivo experiments and medical trials must be performed to establish the therapeutic efficacy and safety of the mucoadhesive gel in human subjects. Examination of the synergistic interest of *Eugenia caryophyllata* and *Cinnamomum verum* with different anticancer herbal or artificial retailers may give insights into combination treatment plans in OSCC. Additionally, mechanistic research directed towards molecular targets for apoptosis induction and tumor growth inhibition could better elucidate the mode of motion of the bioactive compounds. Lastly, improvement of the method to be most efficient for affected person compliance, consisting of sensory traits and person acceptability, would be vital for scientific application.

5. Conclusion

5.1. Summary of Key Findings:

The studies efficaciously designed and examined a mucoadhesive gel with *Eugenia caryophyllata* (clove) and *Cinnamomum verum* (cinnamon) extracts as an effective therapy for oral cancers. The in-vitro tests proved tremendous cytotoxicity against oral squamous cell carcinoma (OSCC) cells, and in-vivo research upheld the efficacy of the gel to inhibit tumor increase. The gel showed excessive mucoadhesion, allowing retention for prolonged intervals and continuous release of the drug at the affected site.

5.2. Significance of the Study:

The outcomes exhibit the promise of herbal bioactive compounds as robust, target site-precise treatment sellers for most common oral cancers. By reducing systemic toxicity and increasing therapeutic efficacy, this system provides a precious alternative to conventional chemotherapy and radiation treatments. Moreover, the mucoadhesive belongings of the gel provides an modern answer for enhancing drug retention in the oral cavity regions.

5.3. Final Thoughts or Recommendations:

Future studies need to involve scientific trials to affirm the safety and efficacy of the formula in human subjects. Optimization of the composition of the gel may be in addition developed to advance the bioavailability and therapeutic efficacy. The investigation on synergistic results of different natural molecules may additionally open up additional contemporary, biocompatible treatment modalities for oral cancers therapy.

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