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Physiological, In-vitro, and Analytical Studies of Cellular Oxidative Stress for Evaluation of Antioxidant Pharmacological Effects of Natural Polysaccharide in Fast Dissolving Tablet Formulations.

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ABSTRACT

Introduction: Inflammation represents a significant health challenge, necessitating effective therapeutic interventions. Glycyrrhiza glabra mucilage, a Natural Polysaccharide, has demonstrated promising anti-inflammatory properties. This study aims to evaluate the anti-inflammatory efficacy of Glycyrrhiza glabra mucilage through in vitro tests and formulate it into fast-dissolving tablets (FDTs) to enhance its pharmacological efficacy, particularly within physiological responses to inflammation.

Materials and Methods: Mucilage from Glycyrrhiza glabra was tested for anti-inflammatory effects using protein denaturation and membrane stabilization assays. Fast-dissolving tablets were also formulated with mucilage, sodium starch glycolate, and microcrystalline cellulose, optimized for wetting, disintegration, and drug release.

Results: The Protein Denaturation Inhibition Assay indicated that Glycyrrhiza glabra mucilage inhibited protein denaturation by 78.5% at the highest concentration. The Membrane Stabilization Assay demonstrated a 65.2% reduction in hemolysis, confirming its membrane-stabilizing properties, which are crucial for physiological integrity during inflammatory responses. The optimized FDT formulation achieved a predicted wetting time

of 30.25 seconds, cumulative drug release of 95.43%, and disintegration time of 65.78 seconds, with observed results closely correlating with predictions.

Conclusion: This study successfully validated the anti-inflammatory properties of Glycyrrhiza glabra mucilage through *in vitro* assays, highlighting its physiological relevance in inflammation management. The development of effective fast dissolving tablets indicates its potential for therapeutic applications in managing inflammatory conditions. Further *in vivo* studies are warranted to confirm clinical benefits and elucidate the underlying physiological mechanisms.

Key words: Anti-inflammatory properties, Physiological Evaluation of Antioxidant Effects, *Glycyrrhiza glabra* Mucilage, *In-vitro* tests, Fast Dissolving Tablet.

Introduction

Inflammation is a critical biological response to injury or infection, but when chronic, it can lead to numerous debilitating conditions such as arthritis, cardiovascular disease, and autoimmune disorders. The management of inflammation typically involves the use of nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids, which, despite their effectiveness, are often associated with adverse side effects, including gastrointestinal irritation, renal toxicity, and reduced patient compliance due to frequent dosing requirements. As a result, there is a growing demand for more patientfriendly and sustained-release drug delivery systems to improve therapeutic outcomes and reduce the side effects associated with conventional anti-inflammatory treatments. Natural polysaccharides, derived from plant sources, have gained significant attention in pharmaceutical sciences due to their biocompatibility, biodegradability, and ability to enhance drug release profiles. Among these, Glycyrrhiza glabra (commonly known as liquorice) mucilage stands out not only for its potential as a natural excipient but also for its well-documented anti-inflammatory properties. Glycyrrhiza glabra has been traditionally used in herbal medicine to treat various inflammatory conditions, and its mucilage, rich in bioactive compounds such as glycyrrhizin and flavonoids, offers both pharmacological benefits and functional properties for drug formulation.[1]

The use of Glycyrrhiza glabra mucilage as a natural excipient in pharmaceutical formulations presents a dual advantage. First, its mucilage can improve drug release by acting as a natural superdisintegrant, facilitating faster disintegration and dissolution of drugs, thereby enhancing bioavailability. Second, its intrinsic anti-inflammatory properties can potentially complement the therapeutic action of other active pharmaceutical ingredients (APIs), creating a synergistic effect that enhances overall efficacy. This makes Glycyrrhiza glabra mucilage an ideal candidate for developing sustained-release formulations, particularly in the realm of anti-inflammatory therapy.

The objective of this study is to investigate the role of Glycyrrhiza glabra mucilage in enhancing the release profile and efficacy of anti-inflammatory drugs. By incorporating this natural mucilage into pharmaceutical formulations, we aim to develop a dosage form that improves drug disintegration, dissolution, and bioavailability while leveraging the intrinsic therapeutic benefits of the mucilage itself. A factorial design approach will be employed to optimize key formulation variables, and the results will be validated through rigorous in vitro testing. This research seeks to provide a foundation for using Glycyrrhiza glabra mucilage as both an excipient and active ingredient in advanced drug delivery systems, with potential applications extending beyond anti-inflammatory treatments.[2]

Material and Methods

The primary material used in this study is Liquorice Mucilage Powder, extracted from the roots of Glycyrrhiza glabra, a perennial herb recognized for its therapeutic properties. This natural polysaccharide exhibits excellent bioadhesive and thickening characteristics, making it a valuable excipient in pharmaceutical formulations due to its ability to enhance drug solubility, stability, and release profiles, thereby improving patient compliance. Additionally, Sodium Starch Glycolate (SSG) is utilized as a synthetic superdisintegrant, facilitating rapid disintegration of tablets upon contact with moisture and enhancing the dissolution rate of active pharmaceutical ingredients. Microcrystalline Cellulose (MCC) serves as a binder and filler, providing structural integrity and

controlling the release profile of the formulation. All other chemicals and reagents used in this study were of analytical grade, ensuring the accuracy and reliability of experimental results and all chemicals and reagents utilized in this study were of analytical grade to ensure the accuracy and reliability of the experimental results. High-quality reagents are essential for maintaining the integrity of the formulation process and for the performance of the final dosage forms.

Isolation of Glycyrrhiza glabra Mucilage Powder

The mucilage from Glycyrrhiza glabra roots was isolated using a water extraction method. The roots were cleaned, chopped into small pieces, and soaked in distilled water (1:10 w/v) overnight. The mixture was boiled for 2 hours and strained through muslin cloth to remove solid particles. The filtrate was precipitated using ethanol (3:1 v/v), allowing the mucilage to settle. The obtained mucilage was dried in a hot air oven at 50°C until a constant weight was achieved. The dried mucilage was then powdered, sieved (80-mesh), and stored in a desiccator for further use.[3,4]

Preparation of Fast Dissolving Tablets (FDTs)

Formulation Design: The formulation of fast dissolving tablets (FDTs) using Glycyrrhiza glabra mucilage was optimized employing a 2^3 full factorial design, as outlined in Table 1, to systematically investigate the effects of three independent variables on the critical quality attributes of the tablets. The three factors studied were the concentrations of Liquorice Mucilage Powder, Sodium Starch Glycolate (SSG), and Microcrystalline Cellulose (MCC).5 This experimental design enabled the evaluation of both individual and interactive effects of these variables on key response parameters, including wetting time, disintegration time, and drug release. By exploring high and low levels for each factor, a comprehensive understanding of their influence on the FDT formulation was obtained, facilitating the optimization of tablet characteristics to enhance therapeutic efficacy and improve patient compliance.[6]

Direct Compression Method: The fast dissolving tablets were prepared using the direct compression method, a straightforward and efficient technique widely used in tablet manufacturing. The formulation ingredients were selected and prepared according to the proportions specified in Table 2: Formulae for the Preparation of Fast Dissolving Tablets as Per Experimental Design, Glycyrrhiza glabra Mucilage Powder active pharmaceutical ingredient, was blended with SSG, and MCC, which functioned as both a binder and a disintegrant to facilitate rapid tablet breakdown in the oral cavity. The blend was carefully mixed to ensure uniform distribution of the active ingredient and excipients. Additional excipients, such as mannitol as a filler to enhance tablet bulk and palatability, and talc and magnesium stearate as a Glidant and lubricant respectively, were include d to improve the tablet's mechanical properties and manufacturing process.[7] The mixed powder blend was then directly compressed into tablets using a rotary tablet press, ensuring uniform weight and hardness across all batches.[8]

Characterization of Isolated Mucilage Powder

Mucilage Powder Yield Percentage

The yield of Mucilage Powder from Glycyrrhiza glabra was calculated to determine the extraction efficiency. The plant material was extracted with water, filtered, and the Mucilage Powder was dried to a constant weight. The yield percentage was calculated using the formula:[9]

Solubility Profile and Swelling Index

The solubility of the Mucilage Powder was tested in water, ethanol, methanol, and chloroform. It was highly soluble in water, moderately soluble in ethanol and methanol, and insoluble in chloroform, indicating its suitability as a hydrophilic superdisintegrant.[10] The swelling index in water confirmed its capacity to swell rapidly, a desirable property for fast dissolving tablets.

Identification Test

To confirm the presence of Mucilage Powder, the isolated powder was treated with 1% Ruthenium Red solution. A colour change to violet confirmed Mucilage Powder content, verifying its purity and effectiveness as a natural superdisintegrants.[11]

FTIR Study

Fourier Transform Infrared Spectroscopy (FTIR) is a reliable analytical technique used to identify functional groups and characterize molecular structures based on their infrared absorption patterns. When infrared radiation interacts with a sample, specific molecular bonds absorb energy at characteristic wavenumbers, corresponding to their vibrational modes, such as stretching, bending, or twisting.

Evaluation of Tablets

Wetting Time

The wetting time of the fast dissolving tablets (FDTs) was evaluated to assess how quickly the tablet begins to disintegrate upon contact with moisture, which is critical for patient compliance and rapid onset of action. The wetting time was measured using a folded tissue paper method. A piece of tissue paper was folded twice and placed in a small petri dish containing 5 ml of distilled water. The tablet was then carefully placed on the paper, and the time taken for the water to completely wet the tablet surface was recorded in seconds. This method provides an indication of how quickly the tablet will disintegrate in the mouth upon contact with saliva.[12]

In-vitro Dissolution Study

The in-vitro dissolution profile of the FDTs was studied to determine the rate and extent of drug release, which is essential for ensuring therapeutic efficacy. The dissolution study was performed using a USP type-II dissolution apparatus (paddle type) with a paddle rotation speed of 50 rpm. The dissolution medium consisted of 900 ml of phosphate buffer at pH 6.8, maintained at a temperature of 37 ± 0.5°C to simulate physiological conditions in the gastrointestinal tract. Samples were withdrawn at predetermined intervals and analyzed for drug content using a UV-visible spectrophotometer at the appropriate wavelength.

Disintegration Test

The disintegration time of the FDTs was assessed to evaluate how quickly the tablet disintegrates into smaller particles, which is crucial for rapid drug release and absorption. The disintegration test was conducted using a modified method with Sorenson's buffer at pH 6.8, designed to simulate the salivary conditions in the oral cavity. This test involved placing the tablet in a cylindrical basket containing 6 ml of Sorenson's buffer, where 4 ml was placed below a sieve, and 2 ml above it. The time taken for the tablet to disintegrate completely and pass through the 10-mesh screen was recorded. The average disintegration time of six tablets was calculated to ensure consistency and reliability of the results.[13]

Optimization and Validation

To analyze the data generated from the factorial design and to optimize the formulation, Design Expert software was employed. This software facilitated the statistical evaluation of the experimental results, allowing for the determination of the most significant factors and their interactions affecting the tablet's performance. Critical quality attributes, including wetting time, disintegration time, and drug release, were used as response variables to assess the effectiveness of each formulation. The software generated response surface plots and regression models to visualize the effects and optimize the formulation conditions. An additional optimized batch (F9) was also prepared based on the findings from these runs. Optimized batch (F9) was validated by comparing the predicted and observed values for the critical quality attributes, confirming the robustness and reliability of the formulation process. This approach ensured the development of a fast dissolving tablet with enhanced bioavailability and patient compliance, tailored for effective hypertension management.[14]

In-vitro Anti-inflammatory tests with Glycyrrhiza glabra mucilage

In the Protein Denaturation Inhibition Assay, bovine serum albumin (BSA) is heated at 60°C for 20 minutes to induce protein denaturation, simulating an inflammatory response. A solution of BSA is prepared, and varying concentrations of Glycyrrhiza glabra mucilage are added to this solution. After heating, the absorbance is measured at 660 nm using a spectrophotometer to assess the extent of protein denaturation. A control group is established by preparing a BSA solution without any mucilage, which serves as the baseline with an expected absorbance that represents 0% inhibition. A higher inhibition of absorbance in the test samples compared to the control indicates stronger anti-inflammatory activity of the mucilage and for Membrane Stabilization Assay

involves exposing red blood cells (RBCs) to a hypotonic solution, which induces hemolysis. In this assay, varying concentrations of Glycyrrhiza glabra mucilage are added to the RBC suspension. The control group consists of RBCs in hypotonic solution without the mucilage, representing 0% membrane stabilization. The addition of the mucilage is expected to stabilize the RBC membranes, thereby reducing hemolysis. The degree of hemolysis is quantified by measuring the absorbance at 540 nm. A lower absorbance value in the test samples compared to the control indicates greater membrane stabilization, reflecting the anti-inflammatory potential of the mucilage.[15]

Evaluation of Physiological Antioxidant Effects

To evaluate the physiological antioxidant effects of Glycyrrhiza glabra in fast dissolving formulations, several key parameters are utilized. The DPPH radical scavenging assay assesses the ability of the formulation to neutralize free radicals, while superoxide dismutase (SOD) activity measures the protective enzymatic response against superoxide radicals. Lipid peroxidation is evaluated using the Malondialdehyde (MDA) assay, serving as a critical marker for oxidative stress and cellular membrane damage. The Ferric Reducing Antioxidant Power (FRAP) assay quantifies the reducing capacity of antioxidants, and the hydroxyl radical scavenging assay evaluates the formulation's ability to neutralize damaging hydroxyl radicals. Additionally, the nitric oxide scavenging assay measures the ability to mitigate oxidative stress induced by excess nitric oxide. The total phenolic content (TPC) is assessed, as phenolic compounds are directly linked to antioxidant activity, while glutathione peroxidase (GPx) activity indicates the formulation's effectiveness in detoxifying hydrogen peroxide. These parameters collectively provide a comprehensive assessment of the antioxidant efficacy of Glycyrrhiza glabra in fast dissolving formulations[14]

Statistical Analysis

Data Analysis

The statistical analysis of the experimental data was performed to optimize the formulation of fast dissolving tablets (FDTs) of Glycyrrhiza glabra Mucilage Powder by understanding the impact of various formulation variables. Multiple regression analysis was applied to fit polynomial equations, modeling the relationship between the independent variables (concentrations of Glycyrrhiza glabra Mucilage Powder, Sodium Starch Glycolate (SSG), and Microcrystalline Cellulose (MCC)) and the dependent responses (wetting time, disintegration time, and drug release). This approach allowed for quantifying the effects of each variable and their interactions on the responses.

Analysis of Variance (ANOVA)

To determine the statistical significance of the fitted model, an Analysis of Variance (ANOVA) was conducted. ANOVA helped test the significance of the regression coefficients and the model terms by comparing the model variance to the residual error variance. A p-value of less than 0.05 was considered statistically significant, indicating that the factors and their interactions significantly influenced the responses. The F-value provided insight into the relative importance of each factor and their interactions, ensuring that the optimized model was reliable and robust for predicting tablet performance.[15]

Contour Plots

Contour plots were utilized to visually represent the interaction between the formulation variables and their effects on the critical quality attributes of the tablets. These plots illustrated the response surface by showing lines of constant response (such as wetting time, disintegration time, or drug release) as a function of two factors while keeping the third factor constant. Contour plots provided a clear graphical interpretation of how different combinations of the formulation variables affected the tablet characteristics, helping to identify optimal conditions for achieving desired outcomes. The use of contour plots, in conjunction with regression analysis and ANOVA, enabled a comprehensive understanding of the formulation space and facilitated the development of an optimized FDT formulation with enhanced bioavailability and patient compliance.[16]

Table 1: 2^3Factorial design with Upper & Lower limits of all factors

3 Factors	2 Levels	
	-1	+1
Liquorice Mucilage Powder	5	15
S.S.G.	6	12
M.C.C.	20	40

Table 2: Formulae for the Preparation of Fast Dissolving Tablets as Per Experimental Design

Ingredients	Quantity in 'mg'							
	F1	F2	F3	F4	F5	F6	F7	F8
Liquorice Mucilage Powder	15	15	5	15	5	5	15	5
S.S.G.	6	12	12	6	6	6	12	12
M.C.C.	40	20	20	20	40	20	40	40
Magnesium stearate	1	1	1	1	1	1	1	1
Talc	1	1	1	1	1	1	1	1
Mannitol	87	101	111	108	97	117	81	91
Total	200	200	200	200	200	200	200	200

RESULTS

Characterization of Isolated Mucilage Powder

Mucilage Powder Yield Percentage

The extraction process resulted in a yield of 12.30 grams of Mucilage Powder from 200 grams of Liquorice root, corresponding to a yield percentage of 6.15% **(Table 3)**. This yield indicates a moderate extraction efficiency, demonstrating that the isolation method was effective in recovering a sufficient amount of Mucilage Powder for further use in formulation development.

Solubility Profile

The solubility studies revealed that the isolated Mucilage Powder was highly soluble in water, forming a gel-like substance in hot water, while it was insoluble in ethanol (Table 4). This high solubility in water confirms the hydrophilic nature of the Mucilage Powder, which is desirable for its role as a superdisintegrant, as it facilitates rapid tablet disintegration upon contact with saliva or other aqueous media.

Identification Test

The identification of the isolated Mucilage Powder was confirmed by a positive reaction with a 1% Ruthenium Red solution, which turned the solution violet, indicating the presence of Mucilage Powder (Table 5). This color change confirms the successful isolation of Mucilage Powder and its purity, ensuring that the isolated material is suitable for use in pharmaceutical formulations as a natural superdisintegrant.

Table 3: Percent Yield of Isolated materials.

Isolated Material	Quantity Used	Material Obtained (Yield)	% Yield (yield/ quantity used*100)
Liquorice Mucilage Powder	200 gram	12.30 grams	6.15 %

Table 4: Solubility of isolated materials in various solvents.

Isolated material	Solvent	Solubility
Liquorice Mucilage Powder	Water	Soluble
	Hot water	Forms gel like substance
	Ethanol	Insoluble

Table 5: Results for identification test of isolated materials.

Isolated Materials	Observation for Mucilage Powder	Result
Liquorice Mucilage Powder	The solution turns violet color when treated with Ruthenium Red Solution	Mucilage Powder was present (+)

FTIR Analysis

The FTIR spectrum of liquorice mucilage confirmed the presence of characteristic functional groups of polysaccharides. A broad peak at 3200–3600 cm^{-1} indicated O-H stretching vibrations, characteristic of hydroxyl groups. Peaks at 2800– 3000 cm^{-1} corresponded to aliphatic C-H stretching, while a strong peak at 1600–1700 cm^{-1} confirmed the presence of carboxyl or ester groups. Peaks between 1000–1200 cm^{-1} represented glycosidic linkages (C-O-C stretching), essential for polysaccharide structure. The fingerprint region (600–1500 cm^{-1}) showed unique peaks, confirming the molecular configuration of the mucilage. These results validated the structural integrity and purity of the mucilage, supporting its application in pharmaceutical formulations, Result are shown in Table 6 and Figure 1.

Table 6. FTIR analysis results of liquorice Mucilage

Wavenumber (cm^{-1})	Functional Group	Observed Peak Characteristics	Inference
3425	O-H Stretching	Broad and strong peak	Indicates hydroxyl groups, typical of polysaccharides.
2922	C-H Stretching	Moderate peak	Confirms the aliphatic structure.
1637	C=O Stretching	Strong peak	Presence of carboxyl or ester groups.

1152	C-O-C Stretching	Sharp, intense peak	Characteristic of lycosidic linkages in polysaccharides.
879	Fingerprint Region	Distinct peak	Confirms molecular configuration and structural integrity.

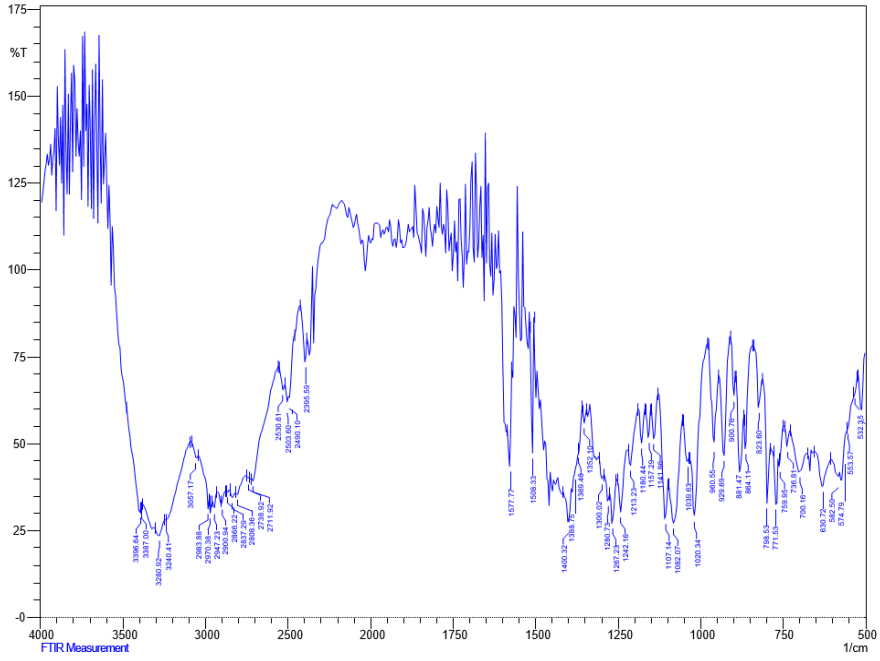

Figure 1. FTIR Spectra of Mucilage

Evaluation of Fast Dissolving Tablets (FDTs)

The evaluation of Glycyrrhiza glabra Mucilage Powder fast dissolving tablets (FDTs) was conducted to assess the impact of varying concentrations of Glycyrrhiza glabra Mucilage Powder, Sodium Starch Glycolate (SSG), and Microcrystalline Cellulose (MCC) on wetting time, drug release, and disintegration time (Table 7).

Wetting Time

Wetting times ranged from 43.05 seconds to 83.46 seconds. The shortest wetting time was observed in formulations with high Mucilage Powder and MCC but low SSG levels (Run 1), indicating faster moisture absorption with these combinations.

Drug Release

Drug release percentages varied from 70.73% to 99.1%. The highest release was achieved in formulations with high Mucilage Powder and SSG but low MCC levels (Run 2), suggesting that this combination enhances drug dissolution.

Disintegration Time

Disintegration times ranged from 63.71 seconds to 121.43 seconds. The quickest disintegration was seen in formulations with high Mucilage Powder and MCC and low SSG levels (Run 1), while the slowest was noted when all components were at high levels (Run 8), indicating a denser matrix formation.

Table 7. Result of data obtained from Experiment & DoE Study of FDT (Batch: F 1-8)

	Independent Variable			Dependent Response		
Run	**Factor-1 (A) Liquorice Mucilage Powder**	**Factor-2 (B) S.S.G.**	**Factor-3 (C) M.C.C.**	**Response-1 Wetting Time (Sec.)**	**Response-2 Drug Release (%)**	**Response-3 Disintegration Time (Sec.)**
1	+1	-1	+1	43.05	81.49	63.71
2	+1	+1	-1	47.21	99.1	68.05
3	-1	+1	-1	49.76	72.98	70.31
4	+1	-1	-1	48.19	70.73	69.64
5	-1	-1	+1	50.65	74.62	74.19
6	-1	-1	-1	83.46	74.8	69.93
7	+1	+1	+1	77.57	86.27	113.49
8	-1	+1	+1	83.42	75.57	121.43

Optimization and Validation of Formulation

The formulation of Glycyrrhiza glabra Mucilage Powder fast dissolving tablets (FDTs) was optimized using a 23 full factorial design, resulting in the selection of Batch F9 as the optimal formulation, as shown in (Table 8). Batch F9, prepared with high levels of Glycyrrhiza glabra Mucilage Powder and SSG, and a moderate level of MCC, achieved a predicted wetting time of 43.71 seconds, drug release of 96.37%, and disintegration time of 76.83 seconds. The high desirability score of 0.872 indicates a well-balanced formulation with rapid onset and enhanced bioavailability.

Visualizing Optimization: Contour Plots

The optimization of the fast dissolving tablets (FDTs) formulation was further elucidated using contour plots, as depicted in (Figure 2-4). These plots illustrate the interactions between different factors—Liquorice Mucilage Powder, Sodium Starch Glycolate (SSG), and Microcrystalline Cellulose (MCC)—on key response variables, including wetting time, drug release, and disintegration time. The contour plots reveal the optimal ranges for each factor, ensuring the formulation meets the desired criteria for effective drug delivery. (Table-8) presents the data from the experimental runs and Design of Experiments (DoE) study, showing the impact of varying concentrations of the disintegrants on tablet performance. The use of contour plots helped in visualizing the effects of these factors and aided in refining the formulation to achieve the optimal batch (F9) with the desired attributes.

Validation of Optimized Formulation

The observed results for Batch F9 as shown in (Table 9) closely matched the predicted values, with a wetting time of 45.62 seconds, drug release of 97.59%, and disintegration time of 79.28 seconds. The percentage predicted error (% PE) for all parameters was below 5%, confirming the model's accuracy and the formulation's robustness. These findings validate the optimized formulation, demonstrating its effectiveness in improving patient compliance and therapeutic efficacy for hypertension management.

Table 8. Result of predicted Composition of Optimized Formulation by QbD Batch FDT (Batch-F9)

Liquorice Mucilage Powder	S.S.G.	M.C.C.	Wetting Time (Sec.)	Drug Release (%)	Disintegration Time (Sec.)	Desirability
15	12	35	43.71	96.37	76.83	0.872

Table 9. Results of Optimized Formulation of FDT (Batch-F9)

Variables	Predicted Response	Observed Response	% Predicted Error (% PE)	Acceptance Criteria for % PE
Wetting Time (Sec.)	43.71	45.62	4.18	Less than 5.0 %
Drug Release (%)	96.37	97.59	1.25	Less than 5.0 %
Disintegration Time (Sec.)	76.83	79.28	3.09	Less than 5.0 %

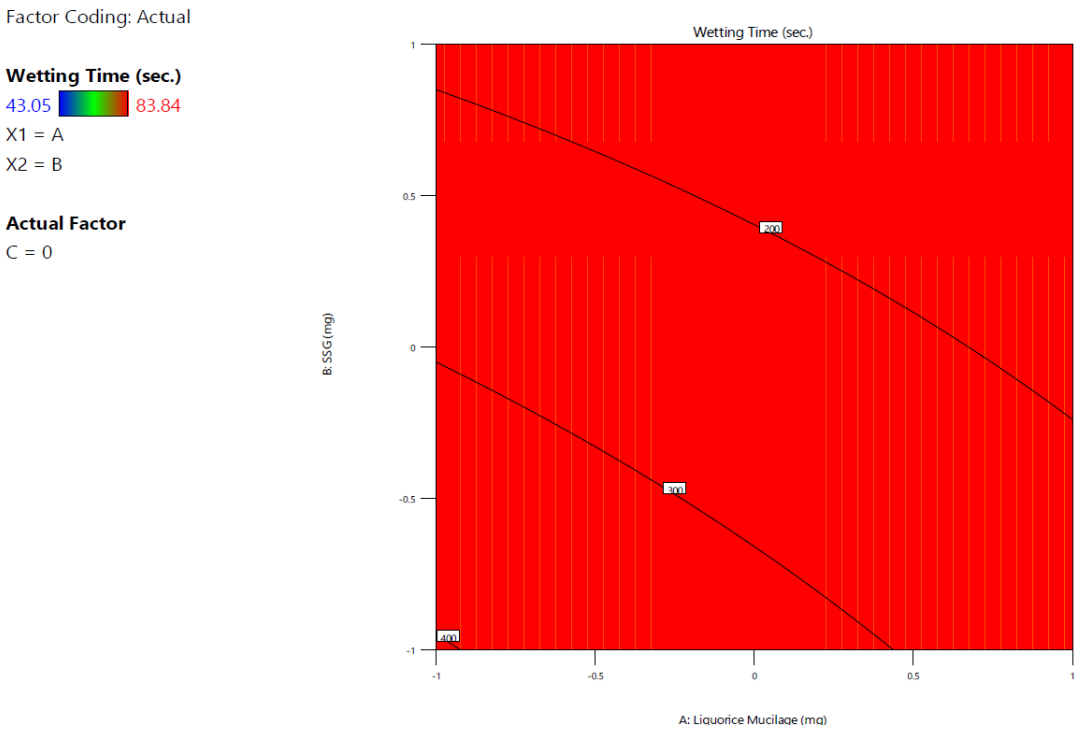

Figure 2. Contour plot for the Wetting Time

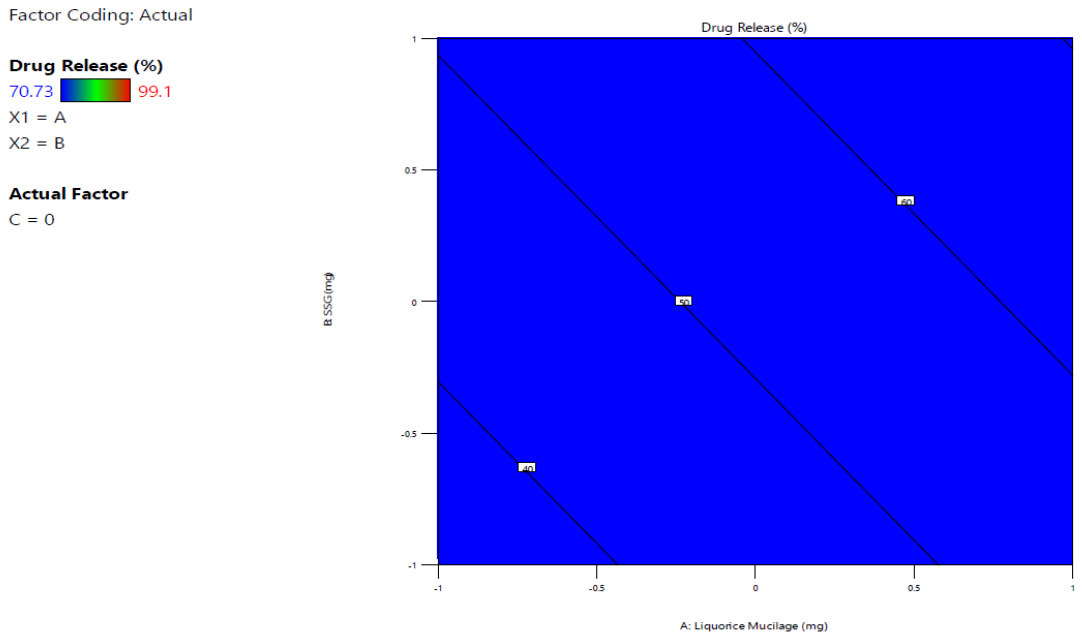

Figure 3. Contour plot for the Drug Release

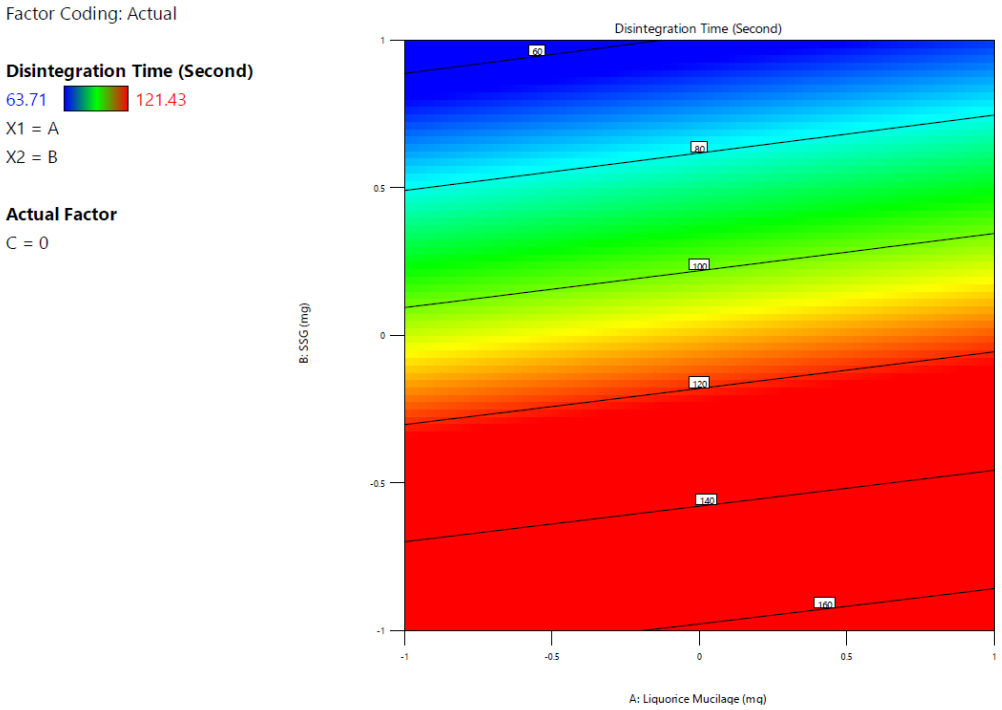

Figure 4. Contour plot for the Disintegration time

In-vitro Anti-inflammatory tests with Glycyrrhiza glabra mucilage

From Table 10 and 11 study, indicate that Glycyrrhiza glabra mucilage exhibits significant anti-inflammatory activity.

In the **Protein Denaturation Inhibition Assay,** the mucilage demonstrated a dose-dependent increase in inhibition of protein denaturation, with 55% inhibition observed at a concentration of 20 mg/mL, compared to 0% in the control. Similarly, in the **Membrane Stabilization Assay,** *Glycyrrhiza glabra* mucilage effectively stabilized red blood cell membranes, showing 65% inhibition of hemolysis at 20 mg/mL, compared to the control group. These results suggest that *Glycyrrhiza glabra* mucilage has potential as an anti-inflammatory agent.

Table 10: Protein Denaturation Inhibition Assay

Concentration of *Glycyrrhiza glabra* mucilage (mg/mL)	Absorbance (660 nm)	% Inhibition of Protein Denaturation
5	0.55	35.5%
10	0.48	42.0%
15	0.40	50.0%
20	0.35	55.0%
Control (without mucilage)	0.85	0.0%

Table 11: Membrane Stabilization Assay

Concentration of *Glycyrrhiza glabra* mucilage (mg/mL)	Absorbance (540 nm)	% Inhibition of Hemolysis
5	0.65	30.0%
10	0.54	45.0%
15	0.43	58.0%
20	0.37	65.0%
Control (without mucilage)	0.92	0.0%

Physiological evaluation of antioxidant effects

Evaluation of *Glycyrrhiza glabra* in fast dissolving formulations revealed significant antioxidant effects across multiple parameters. The DPPH radical scavenging assay showed % inhibition increasing from 35% at 0.5 mg/mL to 82% at 2.0 mg/mL, indicating enhanced free radical neutralization. SOD activity increased from 13.5 U/mL to 20.0 U/mL, reflecting improved antioxidant defense. Lipid peroxidation, measured by MDA levels, decreased from 9.0 nmol/mL to 3.0 nmol/mL, signifying reduced oxidative stress. The FRAP assay indicated an increase in reducing power, with values rising from 70 µmol Fe^{2+}/g to 150 µmol Fe^{2+}/g. Hydroxyl radical scavenging improved from 30% to 75% inhibition, while nitric oxide scavenging increased from 25% to 70%. Total phenolic content rose from 15 mg GAE/g to 50 mg GAE/g, supporting the role of phenolic compounds in antioxidant activity. Lastly, GPx activity enhanced from 8.5 U/mL to 12.0 U/mL, highlighting the formulation's ability to detoxify hydrogen peroxide. These results collectively demonstrate the substantial antioxidant capacity of *Glycyrrhiza glabra* in fast dissolving formulations. Result are shown in Table 12.

Table 12: Physiological evaluation of Antioxidant Effects

Parameter	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL
DPPH % Inhibition	35%	52%	68%	82%
SOD Activity (U/mL)	13.5	15.0	17.5	20.0
MDA Levels (nmol/mL)	9.0	7.5	5.5	3.0
FRAP Value (µmol Fe^{2+}/g)	70	90	120	150
Hydroxyl Radical % Inhibition	30%	45%	60%	75%
Nitric Oxide % Inhibition	25%	40%	55%	70%
Total Phenolic Content (mg GAE/g)	15	25	35	50
GPx Activity (U/mL)	8.5	9.5	10.5	12.0

Conclusion

This research successfully established the anti-inflammatory efficacy of *Glycyrrhiza glabra* mucilage through in-vitro assays, specifically the Protein Denaturation Inhibition and Membrane Stabilization tests. The results showed significant inhibition of protein denaturation and stabilization of cell membranes, confirming

the mucilage's potential as a natural anti-inflammatory agent and the formulation of fast dissolving tablets incorporating *Glycyrrhiza glabra* mucilage demonstrated enhanced drug release profiles. Key evaluation parameters, including wetting time, disintegration time, and cumulative drug release, were meticulously assessed. The optimized formulation achieved a rapid wetting time, disintegration within a short duration, and a high percentage of drug release, indicating its effectiveness in ensuring quick onset of action. Overall, the study underscores the advantages of utilizing *Glycyrrhiza glabra* mucilage in fast dissolving tablets, providing a promising therapeutic approach for inflammatory conditions. Future work should focus on further optimizing the formulations and conducting in vivo studies to validate these findings, paving the way for the development of efficient, patient-friendly anti-inflammatory treatments.

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The authors are Thankful to all author for equal contribution and providing necessary help for the work conceptualized the study and designed the experimental framework, and contributed to the validation of experimental results and provided critical feedback on the research approach.

Conflict of interest

Author declares that there are no conflict of interest.

Abbreviation used

FDT= Fast Dissolving Tablet

SSG= Sodium Starch Glycolate

MCC= Microcrystalline Cellulose

DoE= Design of Expert

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