

FORMULATION, CHARACTERIZATION AND EVALUATION OF GENISTEIN- LOADED GOLD NANOPARTICLE CONTAINING EMULGEL FOR ENHANCED ANTICANCER ACTIVITY

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Abstract: Breast cancer is one of the most common cancers reported worldwide, with about 2.26 million cases reported, according to GLOBOCAN (2020). Approximately 8–9% of breast cancer cases have been found in India, whose prevalence rate continues to increase every year. Conventional methods of therapy suffer from drawbacks such as toxicity and resistance. Genistein-loaded gold nanoparticle emulgel was formulated in this research. Genistein, exhibiting anticancer effects as an active component, has limitations such as low solubility and bioavailability. In this study, Genistein was loaded into gold nanoparticles and these loaded gold nanoparticles were incorporated in emulgel having ideal physicochemical characteristics. Release kinetics indicated the prolonged release of the drug. The antiproliferative effects exhibited by the formulations were evaluated against MCF-7 cells through *in vitro* testing, demonstrating dose-dependent anticancer effects at $IC_{50} = 100 \mu\text{g/mL}$. Thus, the novel formulation was found to appear promising in terms of efficacy.

Index Terms: Gold nanoparticles, Emulgel, Breast cancer, Genistein, Nanosize, Cell Line, MCF-7

1. INTRODUCTION

Breast cancer is a widely occurring cancer across the world. In 2020, worldwide, 2.26 million new breast cancer cases were observed based on the information of GLOBOCAN. India represents many cases in comparison to the world in that, 8-9% breast cancer cases have been diagnosed [1]. Incidence and mortality rates have increased across all age groups in women from 15 to 49 years. Breast cancer has a wide impact on psychosocial well-being causing depression, anxiety, and reduce the quality of life of affected individuals. It may also contribute a large financial burden in terms of high treatment costs, prolonged treatment duration, and productivity losses [2].

Breast cancer staging is based on tumor size, lymph node involvement, and metastasis. Early-stage breast cancer (Stages 0 and I) is localized and has a better prognosis when detected early. Advanced stages (Stages II–IV) involve regional or distant spread. In stage IV, cancer may metastasize to organs such as the lungs, brain, bones, and liver, leading to poorer survival outcomes [3].

Current treatments, including chemotherapy, hormone therapy, and targeted therapy, improve patient outcomes but have limitations such as side effects, damage to healthy cells, drug resistance, high costs, and limited effectiveness in certain subtypes. Triple-negative breast cancer (TNBC) remains particularly difficult to treat due to the lack of targeted therapies [4].

Breast cancer is a heterogeneous disease with different molecular subtypes, each showing varied responses to treatment. In India, delayed diagnosis, limited healthcare access, lack of awareness, and inadequate screening programs contribute to poor outcomes. Therefore, there is a need for safer, more effective, and accessible therapeutic approaches that improve treatment efficacy, reduce side effects, and support early [5].

Genistein (4,5,7-trihydroxyisoflavone), is a naturally occurring phytoestrogen derived from soy isoflavone. The anti-cancer role of Genistein in breast cancer is already known. Also, Genistein shows some more bio-activities, such as inhibiting tumor cell proliferation, estrogen receptor signaling pathway modulation, inducing apoptosis, inhibiting angiogenesis, and so on. Moreover, Genistein has also been known to be involved in cancer-progression related signaling pathways, such as tyrosine kinase activity and cell cycle regulation. Genistein has poor aqueous solubility and permeability across biological membranes and its rapid metabolic degradation when administered orally restricts its clinical use [6].

Nanotechnology-based drug approaches have a promising strategy for increasing the therapeutic activity by overcoming the limitations of anticancer agents. Among the nanocarriers, such as gold nanoparticles, have significant interest due to their unique physicochemical properties, and these also improve the drug stability and targeted delivery by increasing therapeutic activity by reducing off-target

toxicity. The gold nanoparticles also enhance the permeability and retention effect and these also facilitate the accumulation of therapeutic agents in tumor tissues [7].

Emulgel, a hybrid system, is created by trapping an emulsion inside a gel matrix, which creates a biphasic delivery system. It has several advantages for a topical delivery system, mainly for the poorly water –soluble drugs. At the site of application, it enhances the spreadability, drug loading, controlled release, and prolonged residence time. Compared with conventional topical delivery like creams and gels, the emulgel shows more stability and patient compliance [8]. The integration of nanostructured systems such as AuNPs into an emulgel base enhances the therapeutic activity by enabling the transdermal delivery and sustained drug release [9].

The present study introduces the herbal nanoformulation approach involving the development of Genistein-loaded gold nanoparticles-based emulgel for topical applications in breast cancer treatment. It has a promising alternative to the conventional therapies for breast cancer. This formulation is designed to tackle the main drawbacks such as poor drug solubility, systemic toxicity, and resistance to therapy. This research paper emphasizes the innovation of herbal nanoformulation integrating the therapeutic benefits of a natural bioactive compound with the advanced delivery and the versatility of an emulgel system.

2. MATERIALS AND METHODS

2.1 Materials

Genistein was procured from the Bio-molecules India (Khandwa, M.P.), MCF-7 cell line were procured from National Centre cell lines (Pune), Chloroauric acid were procured from Oxford Lab Fine Chem (Maharashtra, India), Triethanolamine was acquired from Merck Chemicals (Mumbai, India), Dimethyl sulfoxide (DMSO) was received from SD Fine (Mumbai) and, Liquid Paraffin, span 80 and Tween 80 Loba Chemie Pvt. Ltd., Propyl glycol, and Carbopol were received from Loba Chemie Pvt. LTD. All chemicals employed were of analytical grade.

2.2 Methods

2.2.1 Preformulation Studies

Preformulation studies are the initial steps of investigation of drug substances, before formulating a dosage form. In preformulation studies, the drug substance is investigated by physical, chemical, and mechanical methods. These studies include the solubility, stability, and partition coefficient. These parameters support understanding the selection of suitable excipients and formulating strategies. It also helps in predicting potential challenges related to drug delivery and bioavailability. It also ensures the compatibility analysis between the API and excipients. It also contributes to optimized processing conditions and storage requirements overall. These studies provide a scientific foundation for developing a safe and effective, and safe dosage form and given as follows [10].

Organoleptic Evaluation

Organoleptic properties are sensory characteristics of a substance that can be evaluated using human senses, including appearance, color, odor, taste, and texture of the drug or formulation. With the assessment of organoleptic properties, it gets easier to initially identify and evaluate the material. Especially for oral and topical dosage forms, it ensures the patient's acceptability. Any changes in the formulation characteristics may indicate instability or degradation. Organoleptic features are essential for maintaining the product quality and uniformity. Therefore, these steps of evaluation are simple but play a major role in the pharmaceutical industry [11].

Fourier Transform Infrared Spectroscopy

Drug–excipient compatibility studies were carried out using Fourier Transform Infrared Spectroscopy (FTIR) to evaluate potential interactions between genistein and the selected excipients. FTIR spectra of pure genistein, individual excipients, and their physical mixtures were recorded in the range of 4000–400 cm^{-1} . The spectra were compared for any significant changes in characteristic peaks, indicating possible chemical interactions or incompatibilities [12].

Solubility

The maximum amount of the substance that can be dissolved in the solvent is defined as the solubility at constant temperature and pressure to form a homogeneous and stable mixture. The shake flask method was used to determine the solubility. Took a known volume of solvent and add an excess amount of solvent, then sealed the flask to prevent evaporation. The flask was shaken continuously at a constant temperature and separated the undissolved particles by filtration [13]. A UV-Visible spectrophotometer (Shimadzu, UV- 1800, Japan), was used to quantitatively determine the drug by constructing calibration curve.

2.2.2 Preparation of Genistein-Loaded Gold Nanoparticles

Genistein-loaded AuNPs were prepared by a facile chemistry approach, varying the initial concentrations of Genistein and HAuCl_4 . Synthesis was performed by changing the previously reported procedure to obtain different sizes of AuNPs and maximize the loading of Genistein on each particle. Conjugates were obtained when 10/20/30mg of Genistein were suspended in 5mL of DMSO (chosen as the medium because Genistein is poorly soluble in water), denoted as F1, F2, and F3, and added to 45mL of boiling solution of 0.1/0.2/0.3 mM of chloroauric acid, respectively. Solutions were stirred and boiled for another 5 minutes until it changes color to violet, brownish-green, or reddish colors, and reactions were completed. Prepared conjugates were cooled down to room temperature and

collected by centrifugation (12,000 rpm, 15min). As-obtained precipitates were cleaned using ethanol to remove DMSO and unbound Genistein via the next centrifugation cycle, and then re-dispersed in ethanol or dried to obtain black powder for further measurements [14]. The formulation table of Genistein-loaded Gold Nanoparticles has been shown in Table 1.

Table 1: Various formulations of Genistein-loaded gold nanoparticles

Formulation	Genistein (mg)	DMSO(mL)	Conc. Of HAuCl ₄ (mM)
F1	10	5	0.1
F2	20	5	0.2
F3	30	5	0.3

2.2.3 Characterization of AuNPs

Particle Size

The average particle size of the synthesized AuNPs was determined by Dynamic Light Scattering (LENOVO Litesizer 500). For deeper penetration, the Nano-range is greater than 100 nm and generally exhibits hydrodynamic diameters intended for topical use. Smaller nanoparticle size contributes to higher surface area, and enhances the drug loading and better distribution within the gel matrix [15].

Polydispersity Index (PDI)

PDI reflects the uniformity of the size distribution within the nanoparticle population. The value of PDI lower than 0.5 indicates homogeneous dispersion, formulation is stable, and reproducible drug release. The PDI value is higher, and particles are aggregated, which can compromise the quality of the formulation [16].

Zeta Potential

Zeta potential (Anton-Paar 001) suggests the charge at the surface and stability of colloidal solutions. If the value of zeta potential is high, typically $\geq \pm 30$ mV indicates adequate electrostatic repulsion between particles, reducing aggregation during storage. Also, the sign of surface charge can influence interaction with skin components and bioadhesion within the emulgel [17].

Field Emission Scanning Electron Microscopy

The FESEM (TESCAN MAIA3 FESEM) technique was used to examine the surface morphology and surface characteristics of nanomaterials at high resolution. It uses a field emission gun to operate and produces a high- focusing, coherent electron beam via field-induced electron emission [18]. F2 formulation was examined because F2 showed the most favorable overall nanoparticle characteristics and good stability.

2.2.4 Formulation and Evaluation of Emulgel

Formulation of gold nanoparticles F1, F2, F3 was utilized for formulation of emulgel EF1, EF2, EF3 respectively on the basis of different concentration of HAuCl₄ (mM).

Step 1 Preparation of gel base: Weighed the required amount of gelling agent (Carbopol 1%W/V), and then soaked into distilled water overnight. After adjusting the pH (6-6.5) by using triethanolamine (TEA) a clear gel was obtained.

Step 2 Preparation of Emulsion:

- For the oil phase: Measure Span 80 and added into paraffin liquid with continuous stirring.
- For the aqueous phase: Measure Tween 80 and added it to distilled water with continuous stirring. After that, oil phase was added to the aqueous phase with continuous stirring. Emulsion was homogenized and allowed to cool at room temperature.

Step 3 Preparation of Emulgel: In a 1:1 mixture, emulsify the emulsion into gel and stirred gently to avoid air entrapment, then pH was adjusted with triethanolamine [19,20]. Formulation table of emulgel (a. Emulsion phase, b. Gel base) is shown in Table 2.

Table 2: Various formulations of emulgel (a. emulsion phase, b. gel base)

a. Emulsion phase

Ingredients	Function	EF1	EF2	EF3
Genistein-loaded AuNPs	API (g)	0.05	0.05	0.05
Liquid paraffin	Oil phase (mL)	2.5	2.5	2.5
Span 80	Oil phase (mL)	1	1	1
Tween 80	Emulsifier (mL)	1	1	1
Propylene glycol	Co-solvent (g)	0.001	0.001	0.001
Aqueous phase	Aq. phase	q.s.	q.s.	q.s.

b. Gel base

Ingredients	Function	EF1	EF2	EF3
Gelling agent	Gelling agent (g)	2	2	2
pH adjuster	pH adjuster (mL)	0.05	0.05	0.05

Physical Examination

Physical examination or visual examination was performed to determine the color and consistency of the emulgel. It also helps to determine the stability and physical acceptability [21].

Homogeneity

The homogeneity of the prepared emulgel was evaluated to ensure uniform distribution of the API and excipients throughout the formulation. A small quantity of the prepared emulgel was visually inspected for consistency, phase separation, and the presence of any lumps. For the microscopic method, a thin layer of emulgel was spread over the glass slide and placed under the microscope to confirm uniform dispersion of the internal phase [22].

pH

The pH was checked by a digital pH meter (DELUXE pH meter, Sti 432) on a logarithmic scale. It measures the acidic or basic properties of an aqueous solution in terms of H^+ ion concentration. pH of the prepared emulgel was determined by using a digital pH meter. The glass electrode was inserted in the emulgel to determine the pH of the formulations. The value of pH for each formulation was calculated as the average of three readings [23].

Extrudability Studies

This test was used to determine the amount of force needed to drive out matter from the tube. It is calculated by the force required to extrude emulgel from the tube. This method was determined to apply shear stress in the region of rheogram corresponding to a shear rate exceeding the yield and exhibits consequent plug flow. In this study, the emulgel from the lacquered aluminum collapsing tube on application of weight in grams required to extrude at least 0.5cm ribbon of emulgel in 10 seconds [24].

Rheological Studies

Viscosity was determined by a Brookfield viscometer (Visco QC 100). The viscosity of emulgel was determined at 25°C using the cone and plate viscometer using the spindle 52, then connect the spindle 52 with the thermostatically controlled circulating water bath [25].

In Vitro Release Studies

In vitro drug release study was performed using a Franz diffusion cell to evaluate the release behavior of the developed formulation. The diffusion cell comprises donor and receptor compartments, which are separated by a suitable membrane, such as a dialysis membrane or excised animal skin. The membrane was hydrated before use by soaking it in receptor medium. The receptor compartment was filled with a suitable medium like phosphate buffer pH 7.4, kept at a fixed temperature of $37 \pm 0.5^\circ\text{C}$ by a water bath, and continuously stirred. The prepared emulgel formulation was carefully placed in the donor compartment over the membrane. Samples were withdrawn at predetermined intervals of time from the receptor compartment and an equivalent volume of fresh medium was added so that sink conditions could be maintained. The withdrawn samples were analyzed by UV spectrophotometry at that specific wavelength. The quantity of drug released was calculated, and a cumulative drug release plot versus time was obtained, which indicated the kinetics of release from the formulation [26]. This will give useful information on the permeation and release behavior of the drug across the membrane.

Cell Line Study for Breast Cancer (MCF-7)

Cell line study was carried out in Aakaar Biotechnologies Private Ltd., Lucknow. Cytotoxicity on the MCF-7 cell line of the formulated samples was carried out by MTT Assay. Cells (10000 cells/well) were cultured in a 96-well plate and incubated in an Air-Jacketed CO₂ incubator (Heal Force- HF90) for 24h in DMEM media (Dulbecco's Modified Eagle Medium-AT149-1L- HIMEDIA) supplemented with 10% Fetal Bovine Serum (HIMEDIA-RM 10432), 1% antibiotics (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C at 5% CO₂. Next day, the cells were exposed to the different concentrations of the formulated samples. Stock solution of the formulated samples was made in water and diluted to achieve different concentrations of the same in incomplete cell culture media (i.e., devoid of Fetal Bovine Serum). Cells with no treatment were used as a control. Cells with no MTT were used as blanks. After incubation for 24h, MTT solution (5 mg/ml) was added to the cell culture and incubated (Air- jacketed CO₂ Incubator-Heal force-HF90) for 2 h. Finally, the culture supernatant was removed, and the cell layer matrix was solubilized using 100 µl of DMSO, and the absorption was read using an ELISA plate reader (iMark, Bio-Rad, USA) at 540 nm. IC₅₀ was calculated using the software GraphPad Prism 6. Under an inverted microscope (Olympus ek2) using a camera (Am Scope digital camera 10 MP Aptima CMOS), images were captured. Results were presented as Mean ± SEM (Standard Error of Mean). 50% inhibitory concentration (IC₅₀) was presented as Mean ± SEM (Standard Error of Mean) [27]. On the basis of the *in vitro* evaluation EF2 was evaluated for cell line study of breast cancer.

Accelerated Stability Studies

Accelerated stability studies were carried out to evaluate the physical and chemical stability of the prepared formulation under stressed environmental conditions. These studies helped to predict the stability of a long time for any pharmaceutical formulation within a short time period. The formulation containing Genistein- loaded gold nanoparticles was stored in tightly closed containers at accelerated conditions of 40±2°C temperature and 75±5% relative humidity for a period of three months, in accordance with International Council for Harmonization GUIDELINE Q1A(R2) for stability testing of new drug substance and products.

During the study, sample withdrawn at predetermined intervals (0,1,2,3, months) and evaluated for important parameters such as appearance, viscosity, pH, drug content, spreadability and then the color consistency and *in vitro* drug release of the formulated emulgel were also observed [28].

3. RESULTS AND DISCUSSION

3.1. Preformulation Studies

Organoleptic properties

The samples were evaluated: Organoleptic properties of Genistein are shown in Table 3.

Table 3: Organoleptic properties of Genistein

Smell	Odorless
Appearance	Pale yellow crystalline powder

Fourier Transform Infrared Spectroscopy

The compatibility study of Chloroauric acid and genistein showed no significant shifts, disappearance or appearance of characteristic peaks, indicating the absence of chemical interaction between the components. The result confirmed good compatibility, indicating their stability for the successful formulation of gold nanoparticles.

Solubility

The solubility of Genistein in various solvents was determined and given in table no. 4

Table 4: Solubility of genistein in different solvents

Solvent / Medium	Solubility (mg/mL)	Solubility Description
Water	0.03	Poorly soluble
Ethanol	5	Soluble
Methanol	4.5	Soluble
DMSO	50	Freely soluble
Alkaline media (pH 9)	10	Enhanced due to the ionization of phenolic groups

Absorption maxima by UV spectroscopy and preparation of a standard curve of Genistein:

A calibration curve was constructed by plotting the graph versus concentration in the range 20- 100 µg/mL at 297 nm. As the concentration increases, the absorbance increases. The regression equation obtained was A= 0.002C+ 0.067, with a correlation coefficient (R²= 0.992), indicating excellent linearity. This calibration curve was used for the quantitative analysis of the drug in subsequent experiments. The standard calibration curve of genistein is shown in figure 1.

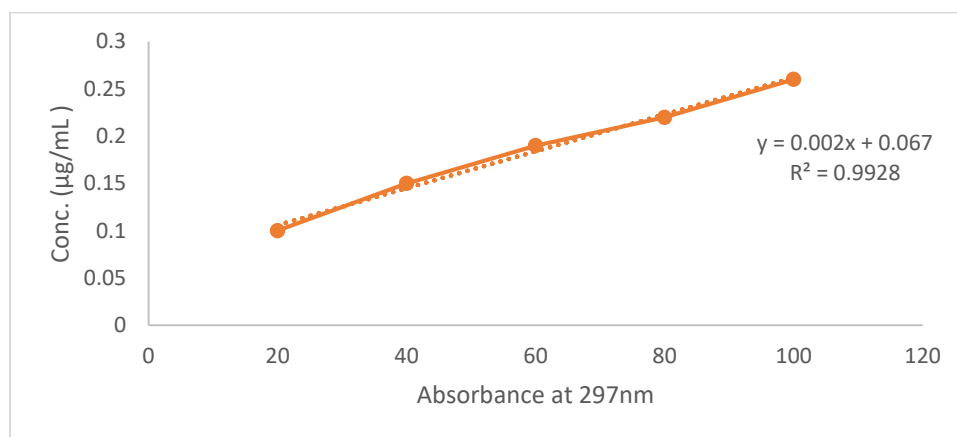


Figure1:Standard calibration curve of genistein

3.2 Preparation of Gold Nanoparticles

Gold nanoparticles were successfully prepared by chemical reduction method, with genistein as the reducing and stabilizing agents and chloroauric acid as the gold precursor.

Particle Size

Particle size analysis confirmed that all three samples were found to be in nanorange. The mean sizes were 499.76 nm, 864.17 nm, 837.82 nm for F1, F2, F3 respectively. F1 exhibited smallest size, it showed efficient particle formation less aggregation and found to possess a larger surface area. While F2 and F3 exhibited slightly larger size.

Polydispersity Index (PDI)

The PDI values of F1, F2 and F3 were 0.255, 0.238, and 0.199, respectively. All the formulations had good particle distribution. The value below 0.3 confirmed that the nanoparticle populations are reasonably uniform. F3 had the lowest PDI value, indicating a more homogeneous and narrowly distributed particle population. These reflected the better control over formulation parameters and improved consistency in nanoparticle synthesis compared with other samples.

Zeta Potential

Zeta potential of the nanoparticles showed values of -0.25 mV, -6.48 mV, and -0.90 mV for F1, F2, and F3 having a surface charge. The presence of a negative charge confirmed that the nanoparticles were stable because the stabilizing group was present on the surface of the negative charge. F2 was found to possess better electrostatic stability due to the highest magnitude of surface charge. Although the absolute value was low, the stabilization occurred through the steric mechanism provided by polymers or capping agents.

Field Emission Scanning Electron Microscopy (FESEM) Analysis

FESEM analysis confirmed that the formulated nanoparticles were spherical, smooth, and uniformly distributed with minimal aggregation. The clear and well-defined morphology indicated successful nanoparticle formation and good stability, making them suitable for a topical emulgel formulation. FESEM analysis of nanoparticles has been shown in figure 2.

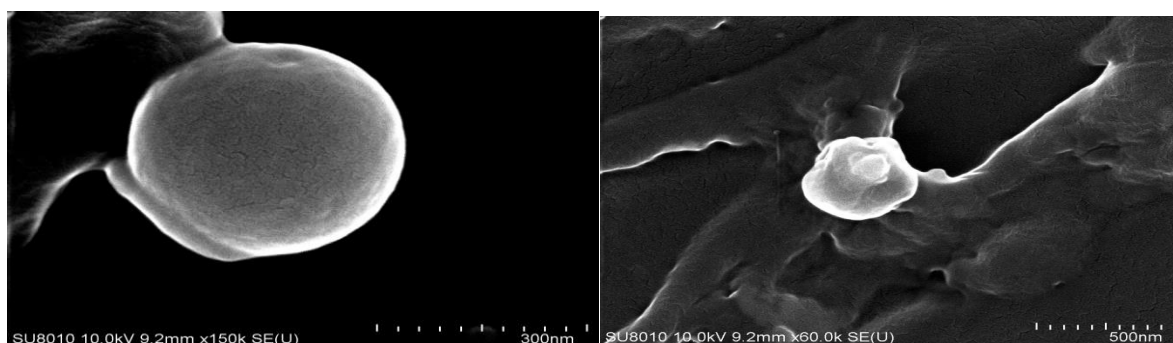


Figure 2: FESEM micrograph of optimized gold nanoparticle formulation (F2)

3.3 Evaluation of Emulgel

A gold nanoparticle-loaded emulgel was successfully prepared, exhibiting a homogenous, appearance and uniform distribution of gold nanoparticles within emulgel matrix.

Physical Examination

The physical examination was done successfully and is shown in Table 5 and visual assessment of physical appearance of prepared emulgel formulation is shown in figure 3.

Table 5: Physical examination of emulgel

Parameters	Results
Color	White
Appearance	Smooth and homogeneous
Texture	Smooth and non-greasy
Consistency	Gel-like
Grittiness	Absent

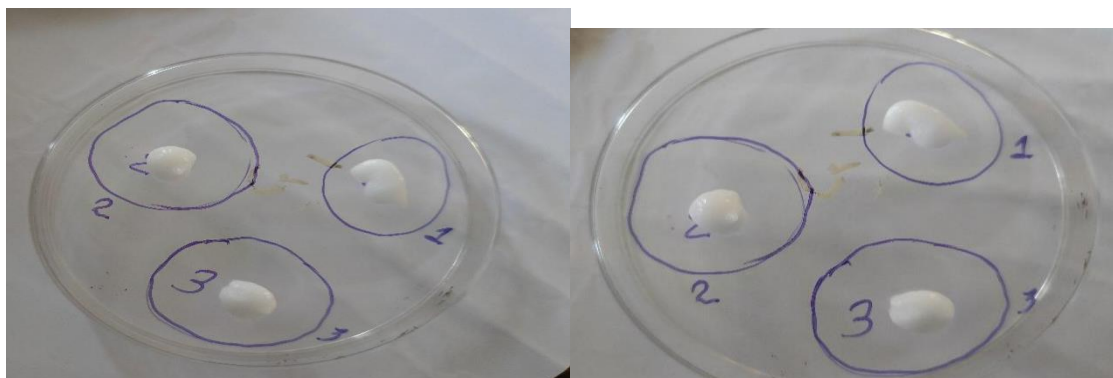


Figure 3: Visual assessment of emulgel formulation (EF1, EF2, EF3)

Homogeneity

The preparation had a uniform, smooth, white appearance and showed no signs of limping, phase separation, or particle formation. Even texture and lack of aggregation suggested even dispersion of the active ingredients and excipients in the formulation.

Therefore, the assessment demonstrated a formulation of good homogeneity and an acceptable physical stability.

Spreadability

The spreadability of the prepared emulgel formulations was evaluated in triplicate to ensure consistency and reliability of the results. Table 6 shows the spreadability of emulgel in cm.

Table 6: Spreadability of Emulgel

Formulation	Reading 1 (cm)	Reading 2 (cm)	Reading 3 (cm)	Mean $\pm$ SD (cm)
EF1	6.15	5.30	5.72	5.72 ± 0.43
EF2	5.30	6.15	5.30	5.58 ± 0.49
EF3	6.15	5.30	5.80	5.75 ± 0.43

All formulations showed a good spreadability, which indicated that the emulgel had a suitable consistency to be applied smoothly on the skin without overexertion. A good spreadability ensures uniform spreading of the drug on the skin surface, and that is a requirement for topical delivery. There was low variation between the readings of these formulations, which implies the uniformity and reproducibility. Overall, the result indicated the developed emulgel suitable for topical application with acceptable physical properties.

pH

The pH of the formulated emulgel preparations was determined in triplicate with accuracy, and the average values of the emulgel formulations are 5.79 ± 0.18 , 5.79 ± 0.33 , and 5.71 ± 0.07 .

Table 7: pH of Emulgel

Formulation	Reading 1	Reading 2	Reading 3	Mean $\pm$ SD
EF1	5.66	5.72	6.00	5.79 $\pm$ 0.18
EF2	5.96	5.41	6.00	5.79 $\pm$ 0.33
EF3	5.66	5.79	5.67	5.71 $\pm$ 0.07

All formulated emulgel preparations exhibited a pH within 5.4 to 6.0 that were found to be suitable for topical use. This range of pH was similar to that of skin (around 4.5-6.5) and suggested that the emulgel will not lead to skin irritation or disrupt the skin barrier function. The standard deviation for formulation 3 was small, and proved to be homogeneous, and the pH of the formulations was found to be stable. Overall, the results confirmed that the prepared emulgel formulations possess an appropriate pH for safe and effective topical delivery.

Extrudability Study

Extrudability of the Genistein-loaded gold nanoparticle emulgel was assessed to determine how easily it would be extruded from the container. It needed an average force of 365 ± 8 g/cm² to extrude a smooth, uniform ribbon of the gel without breaking. Hence, the emulgel formulation has an appropriate consistency for application from the container for easy application by patients. The gel maintained its form after extrusion and easily spread when applied.

Rheological Study (Viscosity)

The viscosities of the prepared emulgel formulation were calculated to determine their consistency to facilitate application on the skin as a drug carrier. The viscosity of EF1 was

13254 mPa·s⁻¹, EF2 was 13262 mPa·s⁻¹, and EF3 was 13248 mPa·s⁻¹. They were consistent with an average viscosity of 13255 ± 7 mPa·s⁻¹. Viscosity of Emulgel is shown in Table 8.

Table 8: Viscosity of various formulations of emulgel

Formulation	Viscosity(mPa s ⁻¹)
EF1	13254
EF2	13262
EF3	13248
Mean $\pm$ SD	13255 $\pm$ 7

In vitro Drug Release

The results showed that all formulations provided a sustained and diffusion-controlled release of the drug from the emulgel matrix. EF2 exhibited the highest drug release, with about 95% of the drug released within 12 hours. EF3 showed a moderate release rate, and EF1 showed the slowest release from the gel matrix. The enhanced release from EF2 may be attributed to better drug solubilization and improved dispersion of the drug within the gel, indicating that EF2 was the optimized formulation. *In vitro* release of formulation is shown in Table 9, and the *in vitro* release profile of emulgel formulations is shown in figure 4.

Table 9: In vitro release of formulations

Time (h)	EFI (%)	EFII (%)	EFIII (%)
0.5	2.1	3.5	3.0
1.0	4.3	7.8	6.5
1.5	6.8	12.6	10.2
2.0	9.4	17.9	14.8
2.5	12.0	23.5	19.6
3.0	14.7	29.8	24.3

3.5	17.5	35.6	28.7
4.0	19.8	40.9	32.5
4.5	22.0	45.3	36.0
5.0	24.3	49.7	39.4
5.5	26.0	53.5	42.0
6.0	27.8	57.8	44.8
6.5	29.5	61.5	47.5
7.0	31.0	62.2	50.0
7.5	32.5	68.8	52.5
8.0	34.0	72.5	55.0
8.5	35.5	76.0	57.2
9.0	37.0	79.5	59.8
9.5	38.5	82.5	61.5
10	40.0	85.5	63.9
10.5	41.5	88.0	69.0
11.0	43.0	90.5	70.9
11.5	44.5	93.0	72.5
12.0	46.0	95.0	73.8

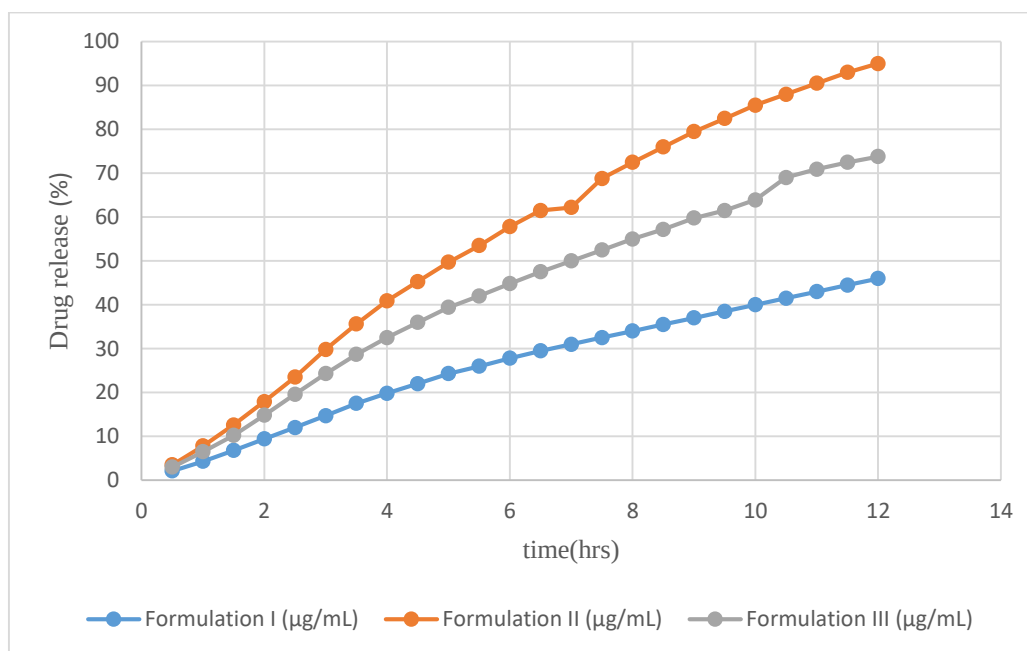


Figure 4: *In vitro* drug release profile of genistein-loaded gold nanoparticles emulgel formulations

Cell Line Study for Breast Cancer (MCF-7)

The cell line study was performed for optimized EF2. The % viable cells decreased as the concentration increased, showing dose-dependent cytotoxic activity. The IC_{50} was approximately 100 μ g/mL, where 50% of cells are inhibited.

$$\% \text{ Viable cells wrt Control} = \text{Absorbance (Test)/Absorbance (Control)} \times 100$$

Where, SD= Standard Deviation

SEM = Standard Error of Mean

According to the results and from the MTT assay, the cytotoxic activity was investigated for cell line MCF-7 after treatment with varying concentrations of EF2, and the results showed a decrease in the percentage of viable cells with an increase in concentration, and the 50% inhibition concentration was found and is mentioned in Table 10, graph shown in figure 5, control and treated group at 1000 μ g/mL shown in figure 6. The cytotoxic activity was studied by MTT assay. A concentration-dependent decrease in % viability of cells was obtained. EF2 showed cytotoxic activity at higher concentrations at 100 μ g/mL, cell viability reduced to $50 \pm 2.5\%$, indicating the IC_{50} value. Further increase in the concentration to 1000 μ g/mL resulted in a reduction in viable cells to $15 \pm 1.5\%$.

Table 10: *In vitro* cytotoxicity results of F2 formulation against MCF -7 cells.

Sample Concentration (μ g/mL)	%Viable Cells (Mean $\pm$ SD)	SEM
0	100 $\pm$ 5.0	3.29
10	90 $\pm$ 4.2	2.70
25	82 $\pm$ 3.5	1.60
50	70 $\pm$ 3.0	1.30
75	62 $\pm$ 2.8	1.00
100	50 $\pm$ 2.5	0.85
250	35 $\pm$ 2.2	0.60
500	20 $\pm$ 1.8	0.55
1000	15 $\pm$ 1.5	0.50

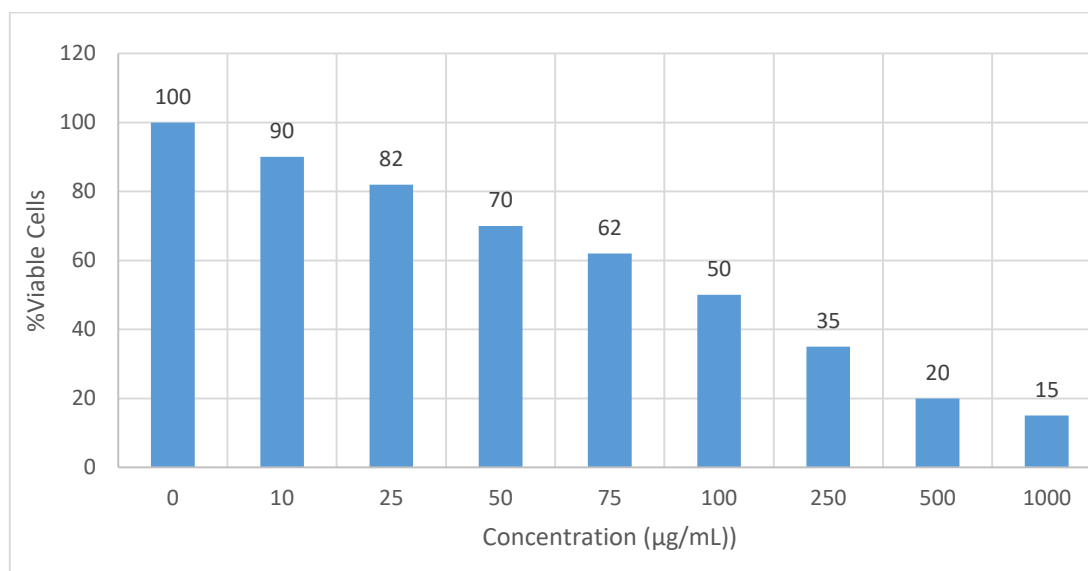
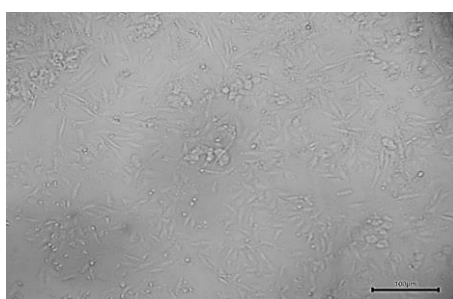


Figure 5: Cytotoxicity assessment of formulation (EF2) at different sample concentration



a. Control group



b. treated group

Figure 6: Morphological comparison of cells: a. untreated control cells, b. cells treated with formulation at 1000µg/mL

Accelerated Stability Studies

An accelerated stability study was carried out to identify the physical and chemical stability of the optimized emulgel formulation under the enhanced conditions of temperature and humidity. The formulations were kept in conditions that are at $40 \pm 2^\circ \text{C}$ and $75 \pm 5\% \text{RH}$ for the study period. Then, at that specific time, for the analyzed parameters like pH, viscosity, spreadability and drug content.

All the parameters were studied, homogeneity of formulation was found to maintain physical parameters throughout the study period showed no sign of phase separation, color change, or lumps.

pH showed only slight fluctuations within the skin-compatible range, signifying the stability of formulation components. Rheological properties like viscosity and spreadability also revealed no significant change. Minimal fluctuation was observed in the drug content of the formulation during the accelerated study, signifying a stable drug moiety with minimal degradation within the emulgel matrix under stress conditions. Overall, good physical and chemical stability was demonstrated, and hence, the formulations could be assured of having a good shelf life.

The results showed the formulation remained physically stable with no significant changes in color, phase, separation, and consistency throughout the study period. The pH values showed only minimal changes within the acceptable range for topical application. There was no significant deviation observed in the drug content and viscosity of the formulation from its initial values, which means there is good stability of nanoparticles in the emulgel matrix.

It proved that the developed composition will show enough stability at storage under acceleration, and it was predicted that it will have a long shelf-life under room conditions.

3.4 DISCUSSION

The current research endeavor was about the development of a Genistein-loaded gold nanoparticle emulgel for topical breast cancer treatment. Preformulation studies established that Genistein is purely formulated, but due to low aqueous solubility, it needs a nanocarrier. UV studies showed that its maximum absorbance at 297nm, with a good correlation coefficient ($R^2 = 0.97$). Particle size

analysis depicted the small nanoparticles with an accepted distribution, and zeta potential values supported a stable system. FESEM studies showed the morphology of spherical particles and less aggregation. Good homogeneity, smooth texture, optimum spreadability, and skin-compatible pH were recorded for the formulated emulgel. The *in vitro* release profile demonstrated the sustained release of the drug from the formulations, with the maximum release being obtained from EF2. The cytotoxicity assay conducted using the MCF-7 breast cancer cell line clearly revealed that a concentration-dependent decrease in cell viability was observed with a measured IC₅₀ value of approximately 100 µg/mL, showing that gold nanoparticles retain the biological activity of the Genistein and demonstrate therapeutic anticancer potential. No significant change in the stability of the formulation was recorded.

4. CONCLUSION

Genistein-loaded gold nanoparticle embedded emulgel for the topical treatment of breast cancer has been developed and characterized in the present study. Incorporation of the nanoparticles in the formulation showed the improvement of drug solubility, stability, and delivery potential. Prepared emulgel was found to have a homogenous appearance, optimum viscosity, high spreadability and pH within skin compatibility range thereby, proving its potential for the topical administration. It demonstrated sustained release of genistein with promising cytotoxicity activity towards the MCF-7 breast cancer cell line with an IC₅₀ value of approximately 100 µg/mL. Stability studies conducted at enhanced conditions for the stated period also showed the good stability of the formulation. Overall, the formulation showed efficient delivery, sustained release of Genistein, significant biological activity, and hopefully, would serve as a new mode of drug delivery for topical treatment of breast cancer with minimized side effects, as its systemic effect is considerably low.

5. FUTURE PROSPECTIVE

The formulated Genistein-loaded nanoparticle emulgel will be characterized *in vitro* to determine its therapeutic efficacy and safety to justify *in vivo* studies and to ultimately consider for human use in the treatment of breast cancer. The formulation can be further improved for effective drug delivery and a target-specific drug release mechanism. Moreover, the use of this nanocarrier for the delivery of poorly water-soluble anticancer drugs can be utilized.

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AUTHOR CONTRIBUTIONS

Nikita Saini: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Validation, Visualization, Writing – original draft.

Dr. Rishu Yadav: Conceptualization, Methodology, Supervision, Project administration, Resources, Validation, Writing – review and editing.

Mona Pipali: Investigation, Data curation, Writing – review and editing.

All authors contributed to the study, critically reviewed the manuscript, and approved the final version for publication.

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