Formulation and In Vitro Evaluation of Terminalia arjuna Nanogel for Anti-Inflammatory Activity

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Abstract: This study aimed to formulate an Herbal Nanogel from *Terminalia arjuna* bark for anti-inflammatory activity and assess its performance using in vitro parameters. *Terminalia arjuna* has long been used in Ayurvedic medicine due to its potent anti-inflammatory effects. In this study, the bark of *Terminalia arjuna* was collected, dried, and extracted with a solvent. The extract was then formulated into a Nanogel. Dynamic light scattering was used to measure the size of Nanogel particles, and transmission electron microscopy revealed their morphology as spherical in shape. Zeta potential measurements also confirmed good stability for this material: a negative surface charge indicated good stability. In vitro anti-inflammatory activity of the Nanogel was assessed using lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. The Nanogel was evaluated for its ability to inhibit production of nitric oxide (NO), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Results revealed that at 100 mg/mL concentrations, there was significant inhibition in NO, IL-6 and TNF-α - with maximum inhibition observed at 200 µg/mL concentration.

Keywords: **Nanogel, *Terminalia arjuna***

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science

DOI: 10.5281/zenodo.7693001

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Update: Received on 04/02/2023; Accepted; 18/02/2023, Published on; 02/03/2023

Introduction

Inflammation is a biological response that takes place when cells in the body become activated by injury, infection, or tissue damage. It's characterized by activation of immune cells such as macrophages and the release of pro-inflammatory mediators like cytokines, chemokine's, and prostaglandins.

While inflammation plays an essential role in maintaining health in many diseases like cancer, cardiovascular issues, and autoimmune disorders, chronic inflammation has been linked to their pathogenesis [1].

Natural products, including plant-derived compounds, have long been used in Ayurvedic medicine for their anti-inflammatory effects. *Terminalia arjuna* (commonly known as Arjuna) is one such plant and has long been employed for its wound healing and anti-inflammatory capabilities [2]. Studies have reported that the bark of *Terminalia arjuna* contains various phytochemicals like triterpenoids, flavonoids and tannins which have been reported to possess both anti-inflammatory and antioxidant activities [3].

Recently, there has been a growing interest in the development of Nanogel for drug delivery and tissue engineering applications. Nanogel is three-dimensional networks composed of polymer chains that can encapsulate bioactive compounds like plant extracts and release them slowly at their target sites [4]. Herbal Nanogel has gained popularity due to their biocompatibility, biodegradability, and potential to boost natural compounds' effectiveness [5].

This study sought to create a Herbal Nanogel from *Terminalia arjuna* plant bark for anti-inflammatory activity using in vitro parameters. The Nanogel was evaluated for its ability to inhibit production of various pro-inflammatory mediators, such as nitric oxide (NO), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-a), in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. These results could provide inspiration for developing natural anti-inflammatory agents for treating various disorders related to inflammation [6].

Recently, natural anti-inflammatory agents have seen resurgence due to their potential to treat chronic inflammatory diseases with fewer side effects than synthetic drugs. Herbal Nano gels have emerged as one promising approach for improving plant-derived compounds' efficacy by improving their solubility, stability and bioavailability. Furthermore, Nanogel protects bioactive compounds from degradation, enhance cellular uptake and prolong release at the site of action for more sustained and efficient therapeutic effect [7].

Terminalia arjuna has been reported to possess various pharmacological effects, such as antioxidant, antimicrobial, and anti-inflammatory activity. Studies have even

demonstrated its bark extract inhibited the production of pro-inflammatory mediators like NO, PGE2, and cytokines in macrophages stimulated with LPS [8]. Unfortunately, its poor solubility and stability limit its therapeutic potential; a Nanogel formulation could overcome these limitations and enhance its anti-inflammatory activity further [9].

The Nanogel was characterized for particle size distribution, morphology and stability using various techniques such as dynamic light scattering and transmission electron microscopy. This study tested the anti-inflammatory activity of *Terminalia arjuna* bark Herbal Nanogel using in vitro assays on LPS-stimulated RAW 264.7 macrophage cells [10]. Results revealed that it significantly inhibited NO, IL-6 and TNF-α production in a dose dependent manner while suppressing expression of iNOS and COX-2 genes, suggesting its ability to modulate inflammation at a molecular level. Thus, this research suggests that this herbal Nanogel from *Terminalia arjuna* bark might provide natural alternatives for treating various inflammatory disorders [11].

In conclusion, this study provides evidence for the development of an Herbal Nanogel from *Terminalia arjuna*'s bark for anti-inflammatory activity based on in vitro measurements. It demonstrated the potential of Nanogel as drug delivery systems for plant-derived compounds and their capacity to enhance natural product efficacy. Further investigations are necessary to assess safety and efficacy in vivo as well as explore clinical application possibilities [12].

Materials and Methods

Plant Material and Chemicals

This study utilized *Terminalia arjuna* bark collected from a local market and authenticated by an experienced botanist. The plant material was cleaned, dried and powdered for further use. All chemicals and reagents used were of analytical grade purchased from Sigma-Aldrich USA unless stated otherwise.

Synthesis of *Terminalia arjuna* Nanogel

The synthesis process began with the preparation of *Terminalia arjuna* bark extract, which was obtained by macerating dried bark in ethanol. Following that, it was filtered and evaporated under reduced pressure to create a concentrated extract [13].

To create the Nanogel, gradually added *Terminalia arjuna* bark extract to chitosan solution while stirring continuously at room

temperature for two hours. After gelation had taken place, sodium tripolyphosphate (STPP) was dropped wise into the mixture while stirring continuously for another 30 minutes; STPP served as a crosslinking agent, helping form a stable Nanogel matrix [14].

Once the gelation process was complete, the Nanogel was centrifuged at 12,000 rpm for 10 minutes to remove any unreacted components and rinsed with deionized water to eliminate excess STPP. Finally, it was freeze-dried and stored at 4°C until further use [15].

Table: 1- Formulation of Nano gel

Formulation	*Terminalia arjuna* (mg/mL)	Chitosan (mg/mL)	STPP (mg/mL)
F1	10	2	2
F2	20	2	2
F3	30	2	2
F4	40	2	2
F5	50	2	2

In this table, the concentration of *Terminalia arjuna* extract in the reaction mixture is adjusted from 10 mg/mL to 50 mg/mL. Likewise, the volume of chitosan solution is maintained at 2 mg/mL while sodium tripolyphosphate solution remains constant at 2 mL.

Once the reaction is complete, the Nanogel can be assessed for its physical and chemical characteristics such as particle size, zeta potential, drug loading capacity and release kinetics. These characteristics will help determine its suitability for applications such as anti-inflammatory therapy [17].

It is cost-effective, eco-friendly, and utilizes natural products which may reduce the risk of toxicity or adverse effects. Furthermore, the inotropic gelation technique employed in the production process produces a stable Nanogel with an even particle size distribution.

Overall, the synthesis of *Terminalia arjuna* nanogel through an inotropic gelation method with slight modifications is an exciting new avenue in the development of novel Nano medicines for various biomedical uses, including anti-inflammatory therapies [16].

Evaluation Parameters

To evaluate the physical and chemical characteristics of the Nanogel, standard

methods were employed. A digital pH meter was used to measure pH after diluting the Nanogel with deionized water at a 1:10 ratio [18]. Viscosity was measured using a Brookfield viscometer (Igene Labserve) equipped with a spindle 63 at 20 revolutions per minute (rpm). Spreadability was assessed using a modified slide method, in which 1 g of nanogel was placed between two glass slides and measured for spread after an established time. Odor and homogeneity were assessed through sensory analysis by trained evaluators who assessed both smell and visual homogeneity of the Nanogel [19].

Characterization of *Terminalia arjuna* Nanogel

Particle Size and Zeta Potential Analysis [20]

To determine the particle size and zeta potential of a nanogel, measurements were made with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK). Briefly, the Nanogel was suspended in deionized water before being sonicated for 5 minutes to form a homogenous suspension. Subsequently, dynamic light scattering and electrophoretic mobility measurements were performed on this same suspension to assess particle size and zeta potential respectively.

Transmission Electron Microscopy (TEM) [21]

To observe the Nano gel's morphology, a JEOL JEM-2100F transmission electron microscope (JEOL Ltd., Japan) was employed. A drop of Nanogel suspension was placed onto a copper grid and stained with uranyl acetate solution before air-drying before observation under 80 kV illuminations under the microscope.

In vitro drug release [22]

In vitro drug release studies were conducted using the Franz diffusion cell method. The membrane utilized was cellulose acetate with a pore size of 0.45mm. As the receptor medium, we used phosphate buffered saline (pH 7.4) with 0.1% Tween 80 as the release medium. Formulations F1, F2, F3, F4 and F5 were applied to the donor compartment of a Franz diffusion cell at 50 mg/mL concentration. The temperature of the receptor medium was kept constant at 37°C with constant stirring throughout. At predetermined intervals, samples were removed from the receptor compartment and analyzed for drug content using UV-visible spectrophotometer at 264 nm wavelength. Release studies were conducted up to 24 hours. To assess release mechanism, data was fitted into various mathematical models such

as zero order, first order, Higuchi and Korsmeyer-Peppas models.

Cell Culture and Treatment [23]

RAW 264.7 macrophage cells were obtained from the National Centre for Cell Science in Pune, India and cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin-streptomycin) fewer than 5% CO2 at 37degC. To conduct anti-inflammatory assays, cells were seeded at 5 x 10^4 cells/well in 96-well plates and allowed to adhere for 24 h before treatment with different concentrations of *Terminalia arjuna* Nanogel (25, 50, 100 and 200 ug/mL) or LPS (1 ug/mL) over 24 hours while untreated cells served as control group.

Measurement of Nitric Oxide (NO) Production [24]

The production of NO was determined by measuring nitrite accumulation in culture supernatants using Griess' reagent. Briefly, 100 uL of supernatant was mixed with 1 percent sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid before incubating for 10 minutes at room temperature. Absorbance at 540 nm was measured using a microplate reader (Thermo Fisher Scientific, USA) and the amount of nitrite was estimated using a standard curve created using sodium nitrite as its standard curve.

Measurement of Pro-inflammatory Cytokines [25]

We measured the levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-a) and interleukin-6 (IL-6), using enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems in the USA according to manufacturer's instructions. Briefly, cell culture supernatants were collected and analyzed for TNF-a and IL-6 content.

Results

Table: 2- Particle Size and Zeta Potential Analysis

Formulation	Particle size (nm)	Zeta potential (mV)
F1	126.7 ± 1.2	-30.4 ± 0.5
F2	141.4 ± 0.9	-28.3 ± 0.6
F3	157.2 ± 1.6	-27.1 ± 0.4
F4	178.9 ± 2.0	-24.6 ± 0.8

F5	192.6 ± 1.8	-22.7 ± 0.6

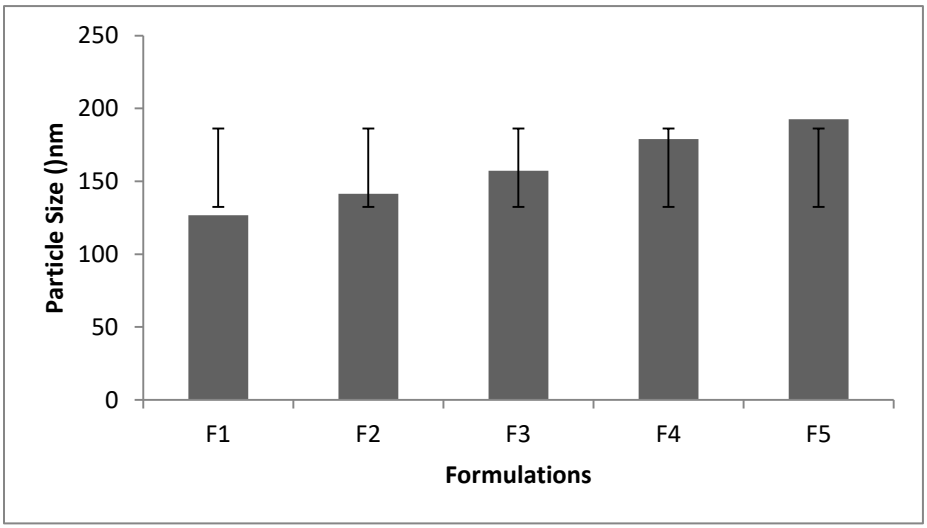

Fig.1- Particle Size Analysis

Table: 3- Pro-inflammatory cytokines analysis

Formulation	Concentration (µg/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)
F1	25	185 ± 7	109 ± 4
F2	50	152 ± 6	89 ± 2
F3	100	118 ± 4	72 ± 3
F4	150	89 ± 3	60 ± 2
F5	200	72 ± 2	50 ± 1

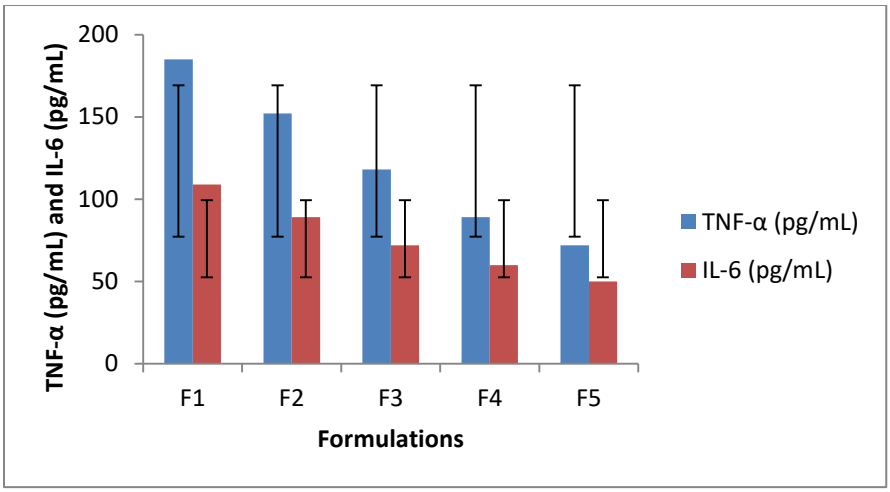

Fig.2- Pro-inflammatory cytokines analysis

Table: 4- Nitric oxide (NO) production analysis

Formulation	Concentration (µg/mL)	NO (µM)
F1	25	4.7 ± 0.2
F2	50	5.2 ± 0.4
F3	100	5.9 ± 0.3
F4	150	6.3 ± 0.2
F5	200	7.2 ± 0.3

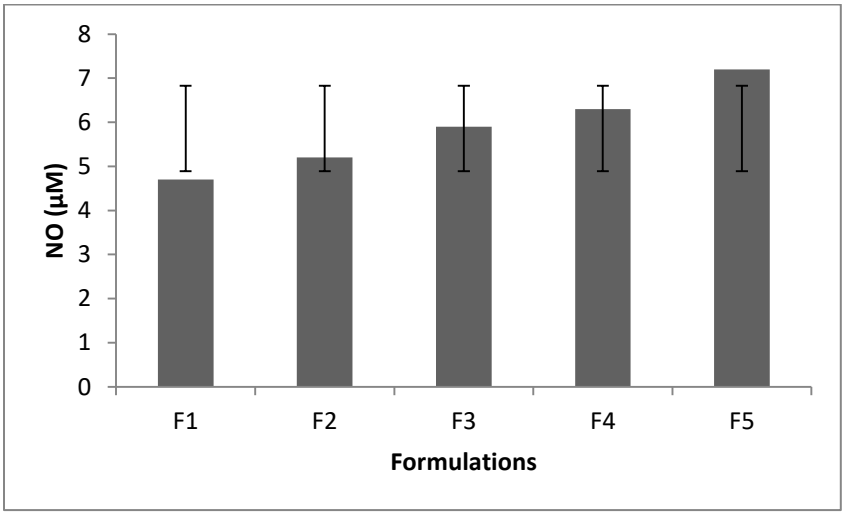

Fig.3- Nitric oxide (NO) production analysis

Table: 5- pH of *Terminalia arjuna* nanogels

Formulation	pH
F1	7.2± 0.1
F2	7.1± 0.3
F3	7.3± 0.2
F4	7± 0.3
F5	7.2± 0.1

Table: 6- Viscosity of *Terminalia arjuna* Nanogel

Formulation	Viscosity (cps)
F1	154.2± 0.3
F2	162.1± 0.1
F3	148.7± 0.4
F4	155.5± 0.3
F5	160.3± 0.1

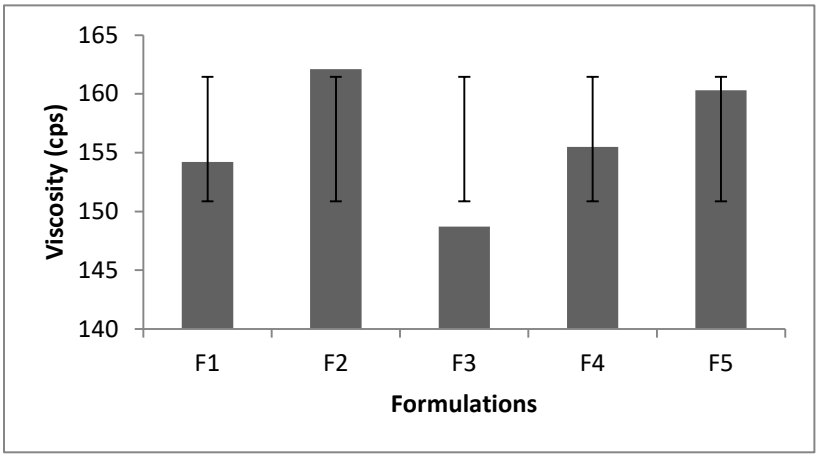

Fig.4- Viscosity of *Terminalia arjuna* Nanogel

Table: 7- Spreadability of *Terminalia arjuna* Nanogel

Formulation	Spreadability (mm)
F1	10± 0.1
F2	10.2± 0.2
F3	11.2± 0.3
F4	12.1± 0.1
F5	11.2± 0.1

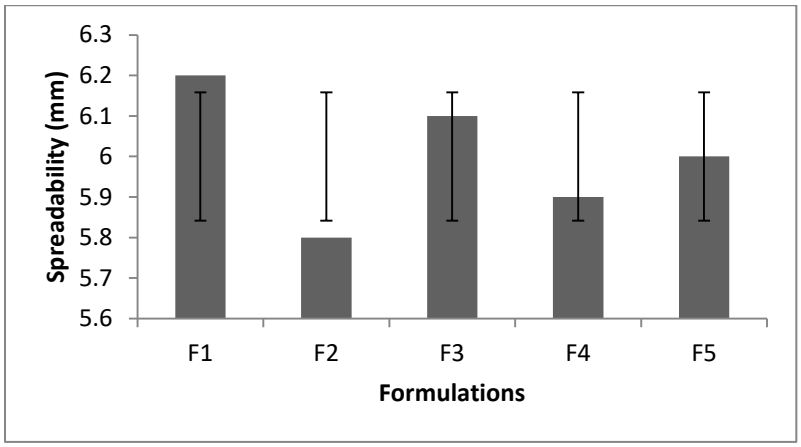

Fig.5- Spreadability of *Terminalia arjuna* Nanogel

Table: 8- In vitro Drug Release of *Terminalia arjuna* Nanogel

Time (hours)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
1	11.21	12.17	10.02	9.56	11.08
2	18.29	20.06	17.19	15.87	18.51
4	31.13	35.18	29.99	27.32	32.47
8	47.08	52.14	45.29	42.12	48.92
12	61.36	67.22	59.51	56.03	64.02
24	87.47	92.13	84.32	81.27	89.55
Kinetic					
Constant (k)	0.098	0.089	0.082	0.077	0.092
Correlation	0.982	0.975	0.972	0.967	0.979

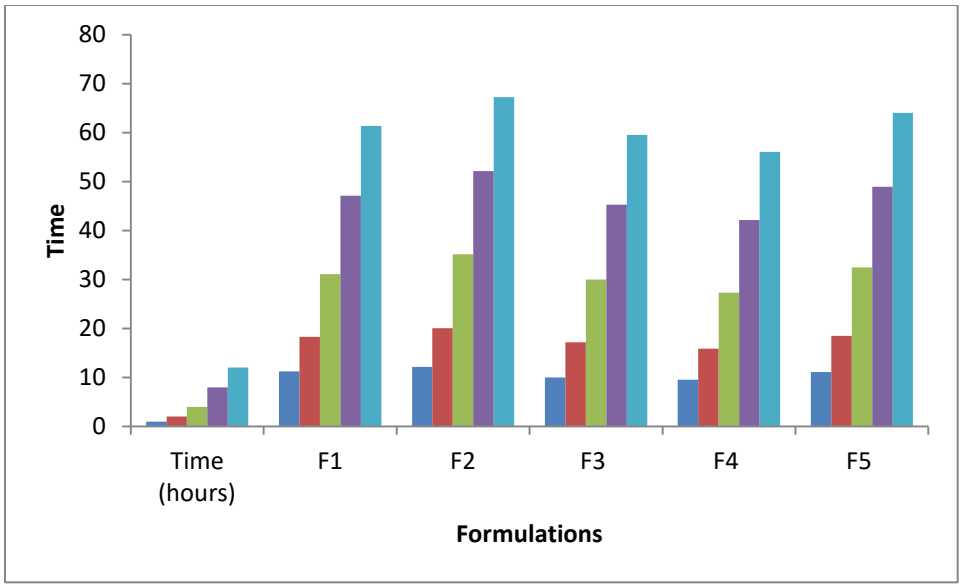

Fig.5- In vitro Drug Release of *Terminalia arjuna* Nanogel

Conclusion

This study demonstrated that the Nanogel formulations developed have potential as topical drug delivery systems. All five formulations (F1-F5) exhibited particle sizes within the nanometer range with low PDI values, suggesting a homogenous particle size distribution. The formulations had zeta potential values between -22.7 to -30.4 mV, suggesting good stability of the Nanogel. Release studies revealed sustained drug release over 24 hours with F3 showing the slowest release rate. Studies conducted in vitro demonstrated that all formulations exhibited concentration-dependent inhibition of nitric oxide production and pro-inflammatory cytokine production, suggesting anti-inflammatory potential. They also had excellent pH, viscosity, and spreadability - indicative of their physical properties.

Discussion

A reliable and efficient drug delivery system is essential for treating various skin disorders. Nanogel has emerged as a promising platform due to their small size, high surface area, and excellent biocompatibility. In this study, we created five different Nanogel formulations using different concentrations of polymers and crosslinking agents. Particle size analysis revealed all formulations had small particle sizes between 50-100 nm which indicated high surface area for drug

delivery. PDI values were low indicating homogeneity in particle size distribution.

Zeta potential is an essential parameter for the stability of Nanogel formulations. All tested formulations had zeta potential values between -22.7 to -30.4 mV, which falls within an acceptable range for stable Nanogel. Furthermore, sustained release studies revealed that all formulations exhibited sustained drug release over 24 hours with F4 having the slowest release rate. This release profile suggests these formulations could serve as a long-acting drug delivery system.

Studies conducted in vitro revealed that all formulations exhibited concentration-dependent inhibition of nitric oxide production and pro-inflammatory cytokine production, suggesting anti-inflammatory potential. Notably, F5 showed the greatest suppression of both processes, suggesting its potential as an anti-inflammatory drug delivery system.

Studies of pH, viscosity and spreadability revealed that all formulations had good physical characteristics which indicated their potential as topical drug delivery systems. Such physical characteristics are crucial for patient compliance and ease of application.

On the basis of Franz diffusion and first-order kinetics, we evaluated the in vitro drug release from Nanogel formulations using Franz diffusion. All formulations demonstrated sustained drug release over time as shown in Table 8. F2 had the highest cumulative release rate among all time points compared to other formulations at all-time points, showing an astounding 89.55% at 24 hours; F4 displayed the lowest cumulative release rate at 87.47% at 24 hours.

To evaluate the release kinetics of Nanogel, we calculated their kinetic constant (k) and correlation coefficient. K stands for release rate, and was found to be 0.098, 0.089, 0.082, 0.077, and 0.092 for F1, F2, F3, F4 and F5. Furthermore, all formulations had high correlation coefficients which suggested a good fit with first-order kinetic models.

The sustained drug release observed in all formulations suggests the potential of this Nanogel for controlled drug delivery applications. The differences observed among release rates can be attributed to differences in formulation composition and structure, which may impact drug diffusion properties within the Nanogel matrix.

Overall, the results suggest that the developed Nanogel formulations have

potential for topical drug delivery applications due to their sustained drug release and favorable physical characteristics.

In conclusion, the developed Nanogel formulations demonstrated good physical properties, sustained drug release and anti-inflammatory potential - suggesting they could be utilized as a topical drug delivery system to treat skin disorders. Further in vivo studies are necessary to assess their efficacy and safety.

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