Formulation and evaluation of Rutin herbal suppositories for the treatment of Inflammatory Bowel Disease

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Abstract

This study focused on formulating rectal rutin suppositories using the heat fusion technique. The formulations incorporated herbal rutin as the active pharmaceutical ingredient (API), with glycerinated gelatin serving as the suppository base. Tween 20 was employed as a solubilizing agent, while methylparaben was included as a preservative. Glycerin and Gelatin were selected as the optimal base components due to their biocompatibility and ability to melt at body temperature. Tween 20, a non-ionic surfactant, was utilized to enhance the solubility of the rutin suppositories, and methylparaben was incorporated to extend the shelf life of the suppositories by preventing microbial contamination.

During the pre-formulation study, rutin was identified as having an acidic nature, which posed a potential challenge for formulation stability and biocompatibility. To address this issue, a phosphate buffer system was incorporated to maintain the formulation within an optimal pH range. This buffering system ensures the physicochemical stability of the formulation while preventing potential degradation of rutin and minimizing the risk of irritation upon administration.

This study conducted a comprehensive evaluation of parameters, including visual inspection, dimensional analysis (length and width), weight variation assessment, liquefaction time determination, hardness test, melting point determination, disintegration time test, drug content analysis study, and in vitro drug release studies. Five formulations were developed, all of which demonstrated satisfactory results. Among these, formulation F5 exhibited the most effective in vitro drug release. To ascertain the formulation's therapeutic efficacy, however, in vivo and clinical research are required.

Keywords – Rutin, Drug, Rectal suppositories, Inflammatory Bowel Disease (IBD), Buffering system, Glycerinated Gelatin

1. Introduction

Suppositories, as solid medicinal preparations, are intended for insertion into body cavities. The term "suppository" originates from the Latin word meaning "to place under". The two main methods of administering medications through the rectum are rectal suppositories and ointments. They can be employed to administer both systemic and localized medications. The general principle is that**,** when inserted into the body, a suppository remains in its solid form initially. However, upon exposure to body temperature and moisture, it gradually melts or dissolves, releasing the active ingredient. This allows the medication to be absorbed efficiently by the surrounding blood vessels, ensuring localized or systemic therapeutic effects. Suppositories were initially used in nursing facilities to administer medications to elderly patients and paediatric patients who were unable to swallow. The rectal drug delivery system offers a more rapid onset of action compared to the oral route. This advantage is primarily due to the avoidance of “hepatic first-pass metabolism”, allowing the drug to be directly absorbed through the rectal mucosa. Consequently, rectal administration enhances systemic drug availability and facilitates faster therapeutic effects.

This administration route offers a practical alternative for delivering pharmaceuticals that induce emesis, cause gastrointestinal irritation, or undergo degradation in the acidic environment of the stomach. Adult rectal suppositories typically weigh around 2 grams, whereas those designed for children are approximately half that weight (1, 2).

A group of long-term inflammatory disorders that are affecting the gastrointestinal system, especially the colon and small intestine, are together referred to as inflammatory bowel disease (IBD).

"Crohn's disease and ulcerative colitis" are its main characteristics. A chronic inflammatory disorder, Crohn's disease can cause irritation and inflammation in any area of the digestive tract. In contrast, ulcerative colitis predominantly impacts the colon (large intestine) and rectum, with inflammation generally affecting only the innermost layer or mucosa.

This condition is characterized by inflammation and the formation of sores. The Crohn’s and Colitis Foundation of America identifies several forms of ulcerative colitis. When the rectum and the distal part of the colon are the only areas affected, the condition is known as ulcerative proctitis. Distal colitis is used if it affects the descending colon and lower sections. Pancolitis is a condition that affects the entire colon.

A long-term inflammatory disease, ulcerative colitis, develops gradually, with symptoms emerging progressively over time. In the initial stages, mild symptoms may arise, including loose stools, abdominal pain, and diarrhoea. As the condition advances from mild to severe stages, patients may exhibit progressive clinical manifestations, including unintended weight loss, persistent fatigue, and a marked decline in appetite. Moreover, disease progression is often associated with gastrointestinal symptoms such as the presence of mucus in the stool and substantial rectal bleeding. Systemic complications may also arise, including pyrexia and anemia.

“An upregulation of pro-inflammatory cytokines like NF-κB, TNF-α, and IL-1β is commonly observed in inflammatory bowel disease (IBD). which contribute to immune dysregulation and subsequent tissue damage. Rutin has been recognized for its potential therapeutic effects in managing IBD due to its anti-inflammatory properties. Specifically, rutin has been shown to reduce myeloperoxidase activity, inhibit TNF-α-induced NF-κB activation in human colon cells, and suppress IL-1β expression, thereby mitigating inflammatory responses associated with the disease” (3, 4,5).

Rutin, also referred to as rutoside, sophorin, quercetin-3-rutinoside, and vitamin P, was first identified in 1842. Sophorin is classified as a citrus flavonoid glycoside and is predominantly present in buckwheat. It is a flavonoid present in a diverse range of plants and is non-toxic, occurring naturally in various plant-based food items, particularly in buckwheat seeds, apricots, tea, cherries, grapes, onions, plums, and oranges. Among different plant species, the highest concentrations of rutin are found in grapes and buckwheat. it is a naturally occurring flavonoid found in various parts of plants, including fruit peels, leaves, flowers, and roots.

Upon reaching the colon, rutin undergoes metabolic transformation by probiotic bacteria, leading to the production of quercetin and other rutin-derived metabolites. These metabolic byproducts contribute to rutin's antioxidant and anti-inflammatory properties (6). The antioxidant properties, effectively scavenging oxidizing agents, including hydroxyl radicals (•OH), superoxide radicals (O_2•−), and peroxyl radicals (ROO•). Therefore, it exhibited various pharmacological effects, including antiallergic, antitumor, antibacterial, antiviral, and antiprotozoal activities. Additionally, rutin has been associated with other therapeutic benefits, such as promoting wound healing, lowering lipid levels, anticancer, managing diabetes, anti-arthritis, nephroprotective activity, and reducing neuroinflammation (3, 7).

Glycerinated gelatin is a widely used base for suppository formulations due to its biocompatibility, ease of melting at body temperatures, and ability to sustain drug release. These properties make it an ideal candidate for developing rectal suppositories. However, the limited solubility and acidic nature of rutin pose significant challenges in formulation development. To overcome these limitations, Tween-20 was incorporated as a solubilizing agent to enhance rutin's solubility; additionally, a phosphate buffer system was employed to maintain the formulation's pH at an optimal level, ensuring its suitability for rectal administration.

This study focuses on the formulation and evaluation of rutin suppositories, with the goal of exploring their potential application in the treatment of IBD. The evaluation parameters include visual inspection study, hardness test (or mechanical strength test), disintegration time determination, dimensional analysis (length and width), weight variation assessment, liquefaction time determination, melting point determination, drug content analysis study, and an in vitro drug release study.

2. Material and Methods

2.1 Material

The materials used in the formulation of rutin suppositories are included. The active pharmaceutical ingredient, Rutin, was procured from Aushadhi Herbal, West Vinod Nagar, Delhi, India (Batch No. AE/03/20124). Methylparaben used as a preservative was procured from Loba Chemi, Mumbai, India (CAS No.99-76-3). Pharmaceutical-grade gelatin powder used as a base and gelling agent was procured from Loba Chemi, Mumbai, India (CAS No. 900-70-8). Glycerin used as a base and plasticizer was procured from Loba Chemi, Mumbai, India (CAS No. 56-81-5). The solubilizing agent Tween 20 were acquired from Meru Chem, Maharashtra, India (CAS No 9005-64-5).

Figure 1: Materials used in the formulation of rutin suppositories

2.2 Method

Rutin-loaded suppositories were formulated using the heat fusion method. Initially, the suppository base was precisely weighed according to the specified formulation and subsequently melted in a water bath (at 70–80°C). Once fully melted, rutin and Tween 20 were incorporated into the molten base and thoroughly mixed using a thin glass rod to ensure uniform dispersion. Following this, methylparaben and distilled water were added to the mixture and stirred until a homogeneous composition was achieved. The resulting formulation was then removed from the water bath and carefully poured into a pre-cleaned and calibrated suppository mold. To facilitate solidification, the molds were placed in a refrigerator at 5°C, allowing the suppositories to harden appropriately. Upon completion of the solidification process, rutin-loaded suppositories were successfully obtained (8, 9).

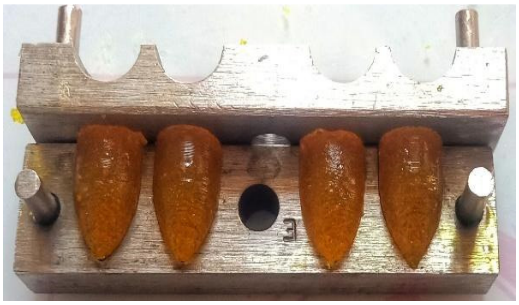

Figure 2: Rutin drug-loaded suppositories

Table 1: The composition of Rutin drug-loaded suppositories

Sl.no	Chemical Used	F1	F2	F3	F4	F5
1.	Glycerin	7ml	7ml	8ml	7ml	7ml
2.	Gelatin	3gm	2gm	3gm	2gm	3gm
3.	Rutin drug (API)	200mg	200mg	200mg	200mg	200mg
4.	Tween 20	0.3ml	0.2ml	0.5ml	_	1ml

5.	Methyl paraben	50mg	50mg	50mg	50mg	50mg
6.	Distilled water	1ml	1ml	1ml	2ml	1ml

Note - An appropriate buffer system was used to maintain the pH of these formulations.

3. Evaluation of Suppositories

3.1 Visual Inspection study

The rutin-loaded suppositories were assessed by randomly selecting 20 suppositories from each batch. These suppositories were then cut longitudinally, and their physical characteristics were examined through direct visual inspection (10).

Table 2: Visual Inspection study

Parameters	F1	F2	F3	F4	F5
Fissuring	Not detected	Not detected	Not detected	Not detected	Not detected
Pitting	Not detected	Not detected	Not detected	Not detected	Not detected
Fat blooming	Not detected	Not detected	Not detected	Not detected	Not detected

3.2 Dimensional Analysis (Length and Width Test)

20 randomly selected rutin-loaded suppositories from each batch. The length and width of each suppository were precisely measured using a vernier calliper and a micro meter screw gauge to ensure precision (11).

3.3 Weight Variation Assessment

20 rutin drug-loaded suppositories were randomly chosen from each batch. Weigh each one separately using the electronic balance device (Contech Instrument Ltd.), then calculate the average weight. The suppository's average weight should not deviate by more than 5%, except for two, which may deviate not more than 7.5% (11, 12).

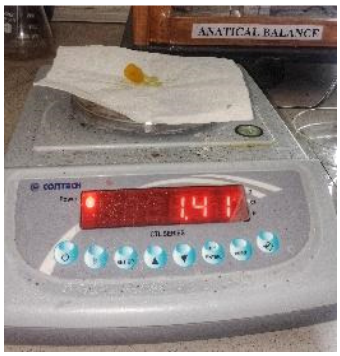

Figure 3: Weight variation test

3.4 Melting Point Determination

- A macro melting range test was conducted for the suppository.
- The assessment of the melting point is a critical factor in determining the release of medicament from suppositories. In this study, the suppositories were placed in a test tube containing 5 mL of phosphate buffer solution (pH 7.2) and held at a constant temperature of 37 ± 0.5°C. The time required for the complete dissolution of the suppository in the medium was recorded (2,13).

3.5 Hardness Test (or Mechanical Strength Test)

Rutin suppositories were randomly selected from each formulation batch and subjected to hardness testing using a calibrated “Monsanto hardness tester”. This assessment evaluates the suppositories’ mechanical strength and their ability to withstand the risks of packaging, handling, storage and transportation (14).

3.6 Liquefaction Time Determination

- This evaluation test measures the time required for rutin suppositories to liquefy under pressure that is similar to rectal pressure in the presence of fluid at a physiological temperature of 37 ± 0.5°C.
- The experiment was performed using a cleaned calibrated burette with a broad opening on one end and a narrow opening on the other. Phosphate buffer (5ml, pH 7.4) was dispensed into the burette and preserved at 37 ± 0.5°C throughout the experiment. A single suppository was introduced into the burette through the broad opening and subsequently guided toward the narrow opening using a thin glass rod. To calculate the liquefaction time, a glass rod was placed on top of the rutin suppository, and the time required for the rod to penetrate the rutin suppository was noted (8, 15).

Note - It should note take more than 30 min.

Figure 4: Liquefaction time test

3.7 Disintegration Time Test

A disintegration test instrument (AE LABS) was used to conduct this test. The formulated rutin suppositories were immersed in 900 mL of phosphate buffer solution with a pH of 7.4 and set up the apparatus to maintained at a normal body temperature of 37±0.5°C (and a speed range of 28–32 cycles per minute). The duration of time required for the suppositories to completely disintegrate and pass through the sieve was noted and considered as the disintegration time (8, 16).

3.8 Drug Content Analysis Study

The drug content assay of the prepared rutin suppositories was conducted using an ultraviolet-visible (UV-Vis) spectrophotometer to quantify the rutin content in the formulation. The analysis was performed in a phosphate buffer (pH 7.4). One suppository was placed in 50 mL of the phosphate buffer and allowed to melt at a temperature of 37 ± 0.5°C. Following complete melting, the formulation was cooled and subsequently filtered using Whatman filter paper. An aliquot of 1 mL from the filtrate was collected and subsequently diluted with phosphate buffer to a final volume of 10mL. Then the diluted sample was analyzed using a UV-visible spectrophotometer (LABTRONICS model LT-2201) at a wavelength of 257 nm (8, 17, 18)**.** The results are tabulated in Table 5.

Figure 5: A UV-Visible spectrophotometer was employed to assess the drug content in the samples.

3.9 In-Vitro Drug Release Profile

The release profile of the drug was evaluated in vitro methods by using a calibrated paddle-type (Type I) dissolution apparatus (LABTRONICS model LT-721) (19). Initially, 900 mL of phosphate buffer (pH 7.4) was prepared and subsequently placed in the dissolution apparatus. A single rutin suppository from each batch was introduced into the apparatus containing the phosphate buffer. The dissolution test was carried out using an apparatus set to rotate at 50 revolutions per minute, while the temperature was consistently held at 37 ± 0.5°C. To assess drug release, 5 mL of the dissolution medium was sampled at 3-minute intervals and immediately replaced with an equal volume of fresh phosphate buffer to ensure sink conditions were preserved (20). The collected samples were analyzed using a UV-Vis spectrophotometer (LABTRONICS model LT-2201) at a wavelength of 257 nm. The % drug release of the rutin suppositories was determined by constructing and utilizing a calibration curve (21). The results are tabulated in Table 6.

4. Result and Discussion

The Pre-formulation studies that were conducted for the rutin drug included morphological characteristics, determination of melting points, solubility assessment, measurement of pKa value, pH analysis, hygroscopicity test, and determination of loss on drying (LOD). The parameter's result of these studies is presented in Table 3.

Table 3: Pre-formulation studies of rutin drug

S.N.	Parameters	Result
1	Colour	Yellow colour
2	Odour	Odourless
3	Taste	Strong bitter
4	Appearance	Very fine powder
5	pKa	6.17
6	pH	
	1% w/v aqueous solution	5-6
	Phosphate buffer system	7-8
7	Solubility	Highly soluble in boiling ethanol
8	Loss on drying (at 105℃)	7.86%
9	Melting point	195℃
10	Hygroscopic	Highly hygroscopic in nature

Rutin suppositories were formulated by employing the "heat fusion method". The formulations comprised a glycerinated gelatin as a base, incorporating Tween 20 as a solubilizing agent and methylparaben as a preservative. An appropriate buffer system was incorporated to maintain

the pH of rutin and stabilize its acidic nature. The suppositories were conducted with various evaluation parameters, including visual inspection study, dimensional analysis (length and width), hardness test (or mechanical strength test), weight variation assessment, liquefaction time determination, melting point determination, disintegration time test, drug content analysis study, and an in vitro drug release study.

The visual inspection confirmed that all prepared suppositories were free from defects, a smooth and glossy surface was observed in the suppositories. Due to the presence of the rutin drug, the developed formulation was observed to be a yellowish-orange colour with a characteristic odour. For dimensional evaluation, randomly selected suppositories were assessed for length and width, with measurements ranging between 1.71 to 1.81 cm in length and 0.79 to 0.91 cm in width, as shown in Table No. 4. The average weight of the suppositories ranged from 0.95 g to 1.41 g, with a deviation not exceeding 5%, which is within the acceptable limit for suppositories.

Hardness testing, a critical parameter for ensuring the stability of suppositories during storage, handling, packaging, and transportation, indicated values ranging between 1.60 to 2.50 kg/cm². These results reflect the ideal hardness properties for the formulations. The melting point test, performed at 37 ± 0.5°C and a pH of 7.2, ensured that the rutin suppositories melted consistently at body temperature to facilitate the release of active ingredients. All formulations melted within 50 minutes, except formulation F4; that value is tabulated in Table 4.

The liquefaction time test measures the time required for suppositories to melt under rectal pressure. All prepared formulations were liquefied in between 1.18 to 2.10 min. It should not take more than 30 minutes. It indicates acceptable liquefaction time for the rutin suppository formulation. The disintegration time test, which assesses the softening, disintegration, and release of active ingredients, showed that all prepared suppositories disintegrated within 10 minutes, which indicates a satisfactory disintegration time for rutin suppositories. These findings are presented in Table 5. Drug content analysis, conducted using a UV-visible spectrophotometer, revealed that all formulations contained rutin within a range of 97% to 99%, indicating an ideal drug content for the prepared suppositories.

The drug release behavior of the five formulations under in vitro conditions is summarized in Table 6. All formulations exhibited more than 50% drug release within 18 minutes. Notably, formulations F3 and F5 saw more than 90% drug release within 21 minutes. The analysis of the drug release profile indicated that formulation F5 exhibited the highest drug release,

reaching a maximum of 95%, as illustrated in Figure 6. Based on these findings, formulation F5 was identified as the most effective formulation among those evaluated. Furthermore, the calibration curve of the best formulation, F5, was observed to have an R^2 value of 0.9953, as represented in Fig. 7. This R^2 value indicates that the prepared dilution process was performed with high accuracy. This result confirms that the formulation is free from impurities. if there are present any impurities that affect the calibration result.

Table 4: Physicochemical characteristics of the formulation

Formulation code	Length (cm)	Width (cm)	Weight variation (gm)	Melting point (37±0.5°C) (min) ± SD
F1	1.71	0.90	1.41	32±0.22
F2	1.73	0.79	0.95	48±0.24
F3	1.80	0.91	1.18	31±0.28
F4	1.75	0.80	1.14	52±0.29
F5	1.81	0.85	1.12	28±0.25

Note - SD: Standard deviation**;** n = 5

Table 6: Drug release profile of rutin suppositories

Time	% Drug released				
	F1	F2	F3	F4	F5

Table 5: Physicochemical characteristics of the formulation

Formulation code	Hardness (Kg/cm^2) ± SD	Liquefaction time (37±0.5°C) (min) ± SD	Disintegration time (37±0.5°C) (min) ± SD	Drug content (%)
3	15.30	14.28	15.70	15.32	15.69
6	23.48	22.52	24.70	25.40	24.74
9	37.82	36.78	38.72	38.92	39.82
12 F1	51.72±0.15	52.15±0.05	51.11	8.15±0.48	42.40	52.10	99.47
15	62.83	63.20	65.50	48.40	64.51
F2	1.95±0.17	1.58±0.43	9.32±0.02	98.37
18	75.23	77.35	81.96	57.50	82.97
21 F3	80.82	1.80±0.16	79.69	1.35±0.13	90.03	8.22±0.08	75.36	95.71	98.43
F4	2.50±0.25	2.10±0.21	9.55±0.07	97.38
F5	1.60±0.11	1.18±0.10	8.29±0.06	99.87

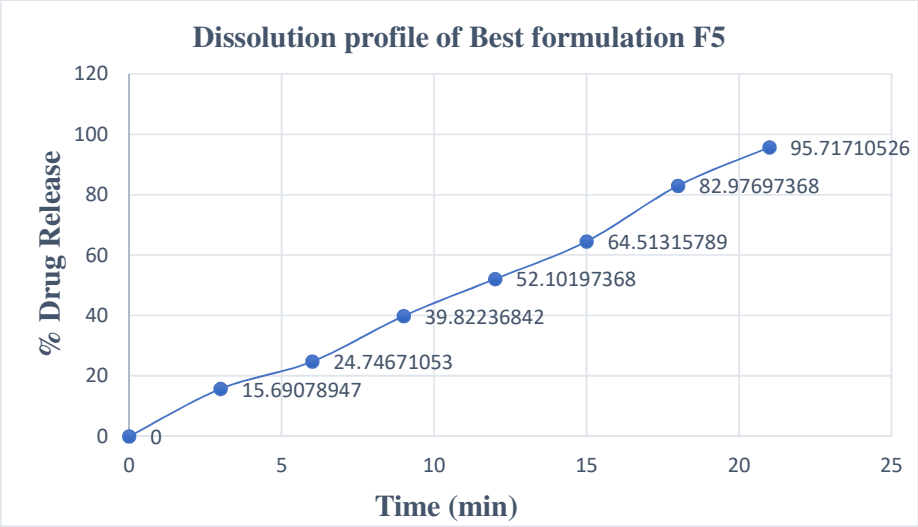

Figure 6: Dissolution profile of best formulation F5

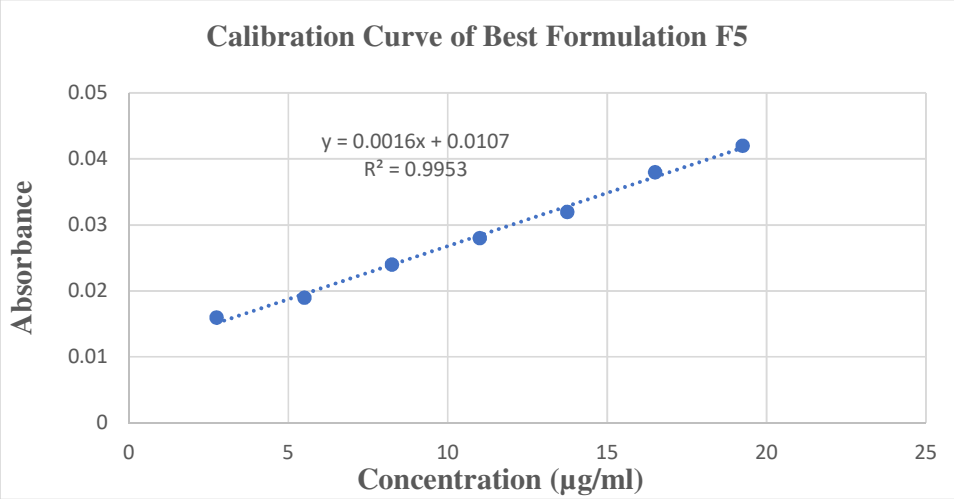

Figure 7: Calibration curve of best formulation F5

5. Future Perspective

The development of rutin-based rectal suppositories for the treatment of IBD holds significant promise due to rutin's potent anti-inflammatory and antioxidant properties. However, several challenges need to be addressed to optimize their formulation and clinical effectiveness. Future research should be focused on the formulation of the following key areas:

- Improving Solubility and Bioavailability: Rutin is noted for poor water solubility and poor bioavailability, which can limit its therapeutic activity. New formulation methods such as nanoencapsulation, solid dispersion, or addition of bioenhancers should be explored to improve its solubility and absorption in rectal tissues. Lipid-based suppositories and self-emulsifying drug delivery systems (SMEDDS) may also enhance the solubilization and permeability of rutin.
- pH Compatibility and Stability: Incompatibility between rutin's optimal pH and the rectal environment may hinder its dissolution and absorption, and the drug may irritate the rectal mucosa. Future research should be focused on pH-modifying excipients in the suppository formulation to ensure adequate drug release and absorption.
- In Vivo and Clinical Evaluations: While in vitro experiments are helpful to know the physicochemical properties and drug release profiles, future research must include

extensive in vivo studies and clinical trials to determine the safety, efficacy, and pharmacokinetic parameters of rutin suppositories.

6. Conclusion

Rutin-based herbal suppositories were formulated using the heat fusion method. The formulations were developed by incorporating rutin as the active pharmaceutical ingredient (API), along with glycerin, gelatin, Tween 20, methylparaben, and a phosphate buffer system to stabilize the acidic nature of rutin and maintain the optimum pH of the formulation. A total of five formulations were prepared by varying the concentrations of excipients. These preparations were tested on various evaluation parameters, including visual inspection study, weight variation assessment, dimensional analysis (length and width), hardness test, melting point determination, liquefaction time determination, disintegration time test, drug content analysis study, and in vitro drug release studies. All five formulations demonstrated satisfactory results, with more than 50% drug release within 18 minutes. However, formulation F5 exhibited the most effective drug release profile, achieving more than 95% within 21 minutes.

In vivo studies using the right animal model are needed to find out more about the bioavailability and therapeutic effectiveness of the suppositories that have been made.

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