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DESIGN AND DEVELOPMENT OF THERMOSENSITIVE RECTAL IN SITU GEL FROM LUFFA ACUTANGULA FRUITS FOR THE TREATMENT OF ULCERATIVE COLITIS

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ABSTRACT

Background: *Luffa acutangula* has good anti-inflammatory and antioxidant activity. Ulcerative colitis has inflamed intestinal mucosal lining with frequent diarrhoea, mucosal, and bloody stools. The rectal administration provides high bioavailability, rapid absorption, and instant therapeutic effect. Conventional rectal formulations may be painful, while insertion and discomfort may also be experienced from the rectum leakage. **Methodology:** *Luffa acutangula* fruit extract was used for the preparation a novel rectal mucoadhesive in situ-gel by using thermosensitive polymers such as Poloxamer 407, Poloxamer 188. HPMC K4M and carbopol 940 are two mucoadhesive polymers that are used to boost the mucoadhesive force and gel strength. The response surface method design was used to optimize the formulation. The formulated in situ gel batches were analysed by gelation temperature, gel strength, gelling ability, gelling time, viscosity and *in vitro* drug release and mucoadhesive strength. **Results and Discussion:** The concentration of poloxamer 407 (15%) and poloxamer 188 (3%) was optimized by design expert. The optimized formulation F10 showed 36.35±0.890 gelation temperature and 94.66±0.57 % cumulative drug release. Drug release kinetics follows Higuchi release model and according to Korsemeyer Peppa's model value of n= 1.1114 indicates supercase -II transport. Gelation temperature of mucoadhesive in situ gel (F10HP2) was found in the range 36.45±0.102°C with 91.37±0.84 % cumulative drug release. Mucoadhesive in situ gel was tested in rat model of ulcerative colitis for 7 days. **Conclusion:** Preclinical study of optimized formulation shows that *Luffa acutangula* fruit extract can stop or prevent further progression of acetic acid induced ulcerative colitis in rat model.

INTRODUCTION

Ulcerative colitis (UC) is known as a chronic, nonspecific, and relapsing form of inflammatory bowel disease (IBD) that primarily affects the mucosal and sub-mucosal lining of the large intestine [1]. The prevalence of the disease in India is estimated to range from 1 to 20 cases per 1,00,000 people, with increasing recognition of the disease in urban areas [2,3].

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The primary etiologies of ulcerative colitis are genetic, heredity, stress, and immunologic factors. Common clinical manifestations of ulcerative colitis include abdominal pain, frequent diarrhoea, mucosal ulceration, and bloody stools [4]. Several medications used in treating UC include immune modulators such as mercaptopurine, azathioprine, methotrexate, adalimumab, infliximab, natalizumab, and certolizumab, as well as anti-inflammatory agents like 5-aminosalicylic acid [6]. The long-term use of these medications can indeed result in drug resistance, adverse reactions, and disease relapse, which not only impact health but also impose substantial financial and psychological burdens on individuals [7,8]. Evidence proved that poor adherence to treatment is a significant barrier to managing ulcerative colitis [3].

The World Health Organization (WHO) estimates that around 80% of the Asian and African population employs herbal drugs, such as curcumin, aloe vera, Boswellia serrata, and many other anti-inflammatory herbals, which are the best therapeutic agents in treating IBD worldwide [5, 9]. Rectal administration involves delivering medication through the rectum, which can be quickly absorbed by the blood vessels in that area [10]. Additionally, it can benefit patients with difficulty swallowing or when gastrointestinal absorption is compromised. The rectal route is a valuable noninvasive drug administration option, which is effective for both local and systemic effects compared to oral routes [11,12]. The rectum is a helpful route for the delivery of some medication because it maintains a fixed concentration of drugs in the plasma [14].

Before administration, the rectal thermosensitive in situ gels are in the sol form. After administration, they undergo an in situ gelation process to convert into gel form at both body temperatures [13,15]. According to the solution-gel transition aspects, quick gelation at the administration site can achieve location adhesion, effective prevention of drug leakage, increased retention period, convenience of drug use, and management of continuous administration [16].

Thermosensitive poloxamers are block copolymers with beneficial properties like good water solubility, surface activity, and reverse thermosensitive gelation properties. When temperature is increased, poloxamers form micelles aggregation with chains of dehydrated poly(propylene oxide) (PPO) and a hydrated poly(ethylene oxide) (PEO) [11,17,19]. Hydroxy propyl Methyl Cellulose (HPMC) K4M and carbopol 940 are essential in situ gels to improve formulations' mucoadhesive and mechanical properties [13,18,20].

Luffa acutangula belongs to the family Cucurbitaceae, called ridge gourd or angle luffa, and is widely growing in India, South Asia, China, Japan, and other parts of Africa. Almost all plant parts, such as fruit, seeds, leaves, and roots, can be used. Different bioactive compounds are present in parts of plants, such as flavonoids, anthraquinones, proteins, saponins, fatty acids, and other phytoconstituents [21,22].

The plant is helpful for treating inflammation, jaundice, spleen enlargement, laxatives, piles, leprosy, ringworm infection, and CNS depression. To improve the current dosage forms, a thermosensitive rectal in situ gel was formulated from *Luffa acutangula* fruit extract [23].

MATERIALS AND METHODS

Collection and authentification of *Luffa acutangula* fruits:

Luffa acutangula fruits were purchased from the local market in the Pune district, Maharashtra, India. The fruits were identified and authenticated at Agharkar Research Institute, Pune. All the analytical-grade chemicals were procured from Tirummala Chemicals, Pune.

Preparation of *Luffa acutangula* fruits extract:

The fruits of *L. acutangula* were chopped into smaller pieces, dried under shade at room temperature, and made into coarse powder. The dried fruit powder (250gm) was packed into the Soxhlet apparatus and continuously extracted with ethanol till complete extraction. After the completion of the extraction cycle, the solvent was evaporated by distillation. The resulting concentrated extract was dried under reduced pressure and stored in an air-tight container for further use [23,24].

Preformulation studies:

Ultra violate spectrophotometric study of ethanolic fruit extract of *Luffa acutangula*:

A stock solution of *Luffa acutangula* extract (1000µg/ml) was prepared by dissolving accurately weighed 10 mg of the extract using ethanol. The final volume was made up to 100 ml using sufficient ethanol. The experimental values of λmax and linearity range values of properly diluted solutions were reported in triplicate [24].

Fourier transform infrared spectroscopy (FT-IR)

FT-IR characterization of pure extract, combination of extract with individual polymer, and extract containing in situ gel was used to monitor any changes in the physical or physicochemical characteristics. Moreover, the IR spectra of blends were recorded after the stipulated period of storage to detect mutual compatibility [25].

Preparation of in situ gel

Formulation of placebo in situ gel

The required quantities of polymers based on the desired concentration were weighed accurately. The polymer concentrations, i.e., poloxamer 407 (5-25% w/w) and poloxamer 188 (1-5% w/w), were added to cold aloe vera juice. Aloe vera juice was used as a vehicle to prepare in situ gel.

The mixture was cooled to 4°C overnight to produce a clear, viscous liquid. The gel strength, gelling temperature, and other characteristics of the prepared in situ gel batches were further assessed [15].

Formulation of rectal in situ gel from ethanolic fruit extract of *Luffa acutangula*

The selected polymers (P407 and P188) were added to cold aloe vera juice (4–5°C), and the extract was added to the polymer mixture and stirred well with a glass rod. The mixture Cooled at 4°C overnight. Cold aloe vera juice was added to fill the volume, and the mixture was transferred to a labeled container and stored in the refrigerator [28, 29]. The quantities of all the ingredients used in the preparation of the rectal in situ gel formulation can be found in Table 1

Design and development of rectal in situ gel from *Luffa acutangula* by using 3^2 factorial designs

In situ gel batches from *Luffa acutangula* fruit extract were developed using poloxamer 407 (5-25%) and poloxamer 188 (1-5%) [26, 27]. Polymer concentrations were decided after a trial of preliminary placebo batches. Preliminary batches (F1, F2, and F3) showed gelation temperature in the 30-36°C range with good gel strength and viscosity. Formulations were optimized by application of 3^2 full factorial designs using design expert 13.0.3.1 version software.

The concentrations of poloxamer 407 (X_1) and poloxamer 188 (X_2) were selected as independent variables. Gelation temperature (Y_1) and Drug release (Y_2) at the end of 7 hrs were taken as response parameters as the dependent variable [29,30]. Coded values and different factorial design batches are as X_1 is low 5%, medium 15%, and high 25%, while X2 is low 1%, medium 3%, and high 5%, respectively.

Formulation of mucoadhesive in situ gel

The in situ gel batch was optimized using a Design Expert to prepare mucoadhesive gel. Medicated gel was found to have weak mechanical strength. For further study, we optimized the concentrations of P407:P188 to 15%w/w:3% w/w, which had significant properties. HPMC K4M and Carbopol 940 were chosen as the mucoadhesive polymers to ensure rectal retention and to improve gel strength and mucoadhesive force [20,26].

The required quantity of Ethanolic fruit extract and mucoadhesive polymers was weighed and appropriately dissolved in aloe vera juice. Poloxamer 407 and poloxamer 188 were gradually added to the cooled above mixture with agitation. After that, the mixture was kept overnight at 4°C to produce a clear, transparent, viscous solution [29,31].

Table 1 Different batches with their respective compositions as per (3^2) factorial design:

Formulation code	Extract (%w/w)	X_1:P407 (%w/w)	X_2: P188 (%w/w)	Aloe vera juice
F1	5%	1	-1	QS
F2	5%	-1	-1	QS
F3	5%	0	1	QS
F4	5%	0	1	QS
F5	5%	-1	-1	QS
F6	5%	0	0	QS
F7	5%	0	0	QS
F8	5%	-1	0	QS
F9	5%	1	0	QS
F10	5%	0	0	QS
F11	5%	1	0	QS
F12	5%	-1	1	QS
F13	5%	0	0	QS
F14	5%	1	1	QS

F15	5%	0	-1	QS	F16	5%	-1	-1	QS

Table 2: Composition and formulation code of medicated mucoadhesive in situ gel

Batch code	Extract (%w/w)	P 407 (%w/w)	P 188 (%w/w)	HPMC K4M (%w/w)	Carbopol 940 (%w/w)	Aloe vera juice
F10HP1	5%	15	3	0.5	-	QS
F10HP2	5%	15	3	0.75	-	QS
F10HP3	5%	15	3	1.0	-	QS
F10CP1	5%	15	3	-	0.5	QS
F10CP2	5%	15	3	-	1.0	QS
F10CP3	5%	15	3	-	1.5	QS

Evaluation of herbal thermosensitive rectal in situ gels

Physical evaluation

The appearance of the formulation was examined for clarity, Colour, and transparency [32,33].

pH determination

The pH of the rectal in situ formulation was determined with a calibrated digital pH meter [39]. The meter is calibrated using standard buffer solutions (pH 6.8), ensuring accurate and reliable readings. The calibrated pH meter electrode was immersed directly into the gel formulation (or diluted sample).

Gelation temperature

The gelation temperature was determined by the tube tilting method. A test tube containing 2 ml solution was sealed with aluminum foil and placed in a water bath at 4°C [35]. The water bath's temperature was progressively raised and observed for 5 min. The gelation temperature was measured when the test tube was tilted 90 degrees and the meniscus stopped flowing [36,37].

Gel strength

8ml of the sol phase of the gel was added to a 10ml glass measuring cylinder and placed in a thermostat at 37°C for gelation [38]. A stainless steel ball-bearing (6 mm diameter and 1.045 g weight) was dropped from a height of 5 cm into the bulk of gel mass to determine gel strength. The gel strength was determined by the distance traveled by the stainless steel ball (mm) within 2 min [39,40].

Gelling time and gelation ability

The sol phase's gelling time (instance) was noted at a constant temperature of 37°C. The time required for phase transition was pointed out after the formulation dropped onto the outer surface of the glass cylinder [40]. The phase characteristics were observed visually, and numerical grades were assigned depending on the time taken for the gel formulation and the time required for the complete gel collapse [41, 42].

Viscosity

The gel phase's viscosity was determined using a Brookfield viscometer (model LVDV II-11) spindle no. S. 62 at 1-5 rpm at Temperature 37°C. The corresponding viscosity (in cp) values were noted [43].

Drug content uniformity

1 ml sol phase was taken and diluted with phosphate buffer (pH 7.4) to adjust the volume to 10 ml. The phosphate buffer (pH 7.4) was used for dilution. The drug content was calculated using spectrophotometry by measuring the absorbance at an experimental value [43,44].

Determination of mucoadhesive strength

A piece of tissue taken from the fundus of a sheep's rectum was used to measure the mucoadhesive force. The tissue fragments were preserved in simulated fluid. A piece of tissue adhered with double-sided adhesive tape to the upper side of a glass vial to measure mucoadhesion. Every mucosa had a diameter of 1.5 cm. For ten minutes, the vials were balanced and maintained at 37°C. One vial containing a tissue section was attached to the balance, and the second vial was fixed to an adjustable pan [45].

Weighed quantities, i.e., 0.1g gel of individual samples of gel formulations, were applied to the base of an inverted glass vial using double-sided adhesive tape to secure the gel in a position [46]. The space between the two vials was adjusted to keep the gel sample attached to the mucosal membrane. The force was applied to both vials for 10 sec. to ensure the gel adhered to the mucosa. The minimal weights added to balance were recorded. The mucoadhesive force was expressed in units of dyne/cm^2 as the following equation.

$$\text{Mucoadhesive force (dyne/cm2)} = \frac{M \times G}{A}$$

Where, M=weight required to add (gm) G=acceleration of gravity (980cm/sec^2); A= tissue exposed area (πr^2) [47]

Dissolution studies (*In vitro*) pH 7.4 Phosphate buffer

In situ gel formulations' in vitro drug release studies used a dissolution apparatus (USP II). The formulations were placed in a semi-permeable bag dipped in a dissolution medium (phosphate buffer pH 7.4). The rotation speed was maintained at 100 rpm at 37°C. A 1 ml sample was taken out at a predetermined interval for further dilution. The samples were diluted and analyzed spectrophotometrically at 263 nm. The experiments were conducted in triplicates [15,43].

Experimental animal study

Sprague Dawley male rats (weighing 200-220 gm, 9-11 weeks old) were purchased from BIOTOX Laboratories, Nashik, Maharashtra, India, and kept under experimental conditions in rat cages with bedding made of paddy husk at a temperature of 23 ± 2ºC with relative humidity 55 ± 10 % under (12 h light and dark cycle) throughout the experiment [49]. All the experimental designs and protocols used in this study were approved after review by the Institutional Animal Ethics Committee (IAEC) of BIOTOX Laboratories, Nashik, Maharashtra, India.

Application of treatment drugs

An extract of Luffa acutangula will be applied intrarectally once a day using a polyethylene tube (2 mm in diameter) for 7 days, with the first dose applied on the first day.

Induction of Ulcerative Colitis

Disease induction was carried out using colon tissue according to the method of Ansari et al. On day 2, after receiving two-hour treatment doses, rats were allowed to fast for sixteen hours (overnight) with unrestricted access to water. Next, pentobarbital sodium (30 mg/kg, IP) induced mild anesthesia [48]. 1 ml of 5% acetic acid (AA) solution was administered by a polyethylene tube (2 mm diameter) inserted through the rectum into the colon up to a distance of 8 cm. Rats were then holed horizontally for 2 min to prevent AA leakage. Rectal washing was done with 0.5 mL saline solution to neutralize the AA solution. Control animals underwent the same procedure using an equal volume of normal saline instead of AA solution [49].

Experimental design

Animals were randomly divided into 5 groups (n=6) and received treatment for 07 days.

1. Normal control= Normal saline (1ml/kg intrarectal) +Normal saline(1ml/kg intrarectal)
2. Disease control= Normal Saline (1ml/kg intrarectal) + AA (1ml/kg intrarectal)
3. Test control= *Luffa acutangula* extract rectal in situ gel (1ml/kg intrarectal) + AA (1ml/kg intrarectal)
4. Standard Control= Mesalamine (300mg/kg) + AA (1ml/kg intrarectal) [48].

Effect of extract of *Luffa acutangula* fruit on % change in body weight

Intrarectal injection of acetic acid in rats experienced colonic inflammatory response, manifesting as loose stool (with or without blood) and weight loss. Weight loss indicates intestinal inflammation's severity and correlates with colitis's histopathological changes. It has been reported that in colitis, increased levels of cytokines such as TNF-α and IL-6 significantly contribute to body weight reduction by releasing appetite-suppressive neuropeptides [52]. Body weight was noted on the 1st and 7th day of the treatment. The following formula will determine the percentage change in body weight [50].

$$\%\ \text{Change in body weight} = \left(\frac{\text{body weight on 7th day}}{\text{body weight on 1st day}}\right) \times 100$$

Effect of extract of *Luffa acutangula* fruit on Disease Activity Index (DAI)

The DAI scores were assessed from 0 (healthy) to 12 (severe colitis). The total scores for rectal bleeding (scores 0-4), stool consistency (scores 0-4), and percentage loss of body weight (scores 0-4) were determined. Mucus from excrement adhered to animal fur, indicating diarrhea, whereas rectal bleeding varied from occult blood to profuse hemorrhage [50,55].

Biochemical assay

The colon tissues were homogenized in 10 mmol Tris–HCl buffer (pH 7.4), and the homogenate was used to measure IL6.

Effect of extract of *Luffa acutangula* fruit on proinflammatory cytokine Interleukin 6 concentration

IL-6 plays a crucial role in ulcerative colitis. It has been reported that inhibiting the IL-6 signal pathway could reduce many pro-

inflammatory factors and improve colitis. The interleukin 6 level was estimated using the ELISA method. The procedure and calculation were carried out as per the instructions given by the manufacturer and expressed as a picomole/mg tissue sample [54, 56].

Histopathological examination:

10% buffered formalin was used to store colons under -80°C, incorporated in paraffin blocks, and stained with hematoxylin and eosin (H&E) (Merck, Darmstadt, Germany). Histopathological evaluation was done under a light microscope. Parameters such as mucosal ulceration, erosion, and edema were graded [51].

RESULTS AND DISCUSSION

Spectrophotometric characteristics of ethanolic fruit extract of *Luffa acutangula*:

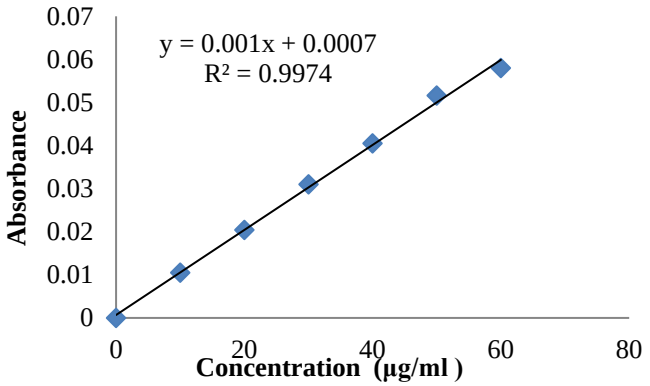

Figure 1: Calibration curve of ethanolic fruit extract of *Luffa acutangula* in pH 6.8 phosphate buffer

Drug-excipient compatibility study

Figure 2 represents the FT-IR characterization of the physical mixture of pure extract, extract, and excipients. The FT-IR spectrum of ethanolic fruit extract gave a broad peak at 3269 and 2621, 1593, 1028, 774, and 707 cm^{-1}, which indicated the presence of O-H, C-H, C=O stretching, and C-H bending, respectively. The FT-IR spectra of physical mixture of poloxamer 407 and 188 peaks at 2881, 1466,1342, 1279, 1241, 1110, 961 cm-1, the spectra of physical mixture of extract and HPMC K40 peaks at 3328, 2918, 2851, 2114, 1707, 1345 cm-1**,** spectra of physical mixture of extract and peaks at 3330, 2918, 2851, 2112, 1963, 1703 cm-1 shows presence of O-H stretching, C-H stretching, C=O stretching, O-H bending and C-H bending respectively [21].

Characteristics of placebo in situ gel formulations:

All placebo in situ gels were clear, colorless solutions. The pH of all the formulations was in the range of 6.6-6.9, near the rectum's pH. The suitable gelation temperature of in situ gel must be 30–36°C for phase transition. Because anus leakage results from the sol phase remaining liquid at physiological temperature at higher temperatures, whereas gelation takes place at room temperature at lower temperatures [15]. Hence, this formulation concentration was used for 3^2 factorial designs of medicated in situ batches.

Characteristics of medicated herbal in situ gel formulations:

All formulations were prepared by following 3^2 full factorial designs.

Physical evaluation

All in situ gel batches were clear and greenish solutions.

Determination of pH

The pH of in situ gel batches was nearing the pH of the rectum (6.6-6.9).

Gelation temperature

The gelation temperature range for in situ gel batches was in the range of 30-36ºC. Both Poloxamer 407 and 188 exhibit thermosensitive properties, and their sol-gel transition temperatures are influenced by concentration. Generally, increasing the concentration of either polymer will raise the gelling temperature, leading to a more stable gel at physiological temperatures.

Gel strength

The distance determined the gel strength traveled (mm) by the stainless steel ball within 2 min through the gel. Adequate gel strength is required to prevent rectum leakage. The distance traveled by the stainless steel ball is inversely proportional to the gel strength, as the lower the distance traveled, the higher the gel strength [30, 31].

Gelling time and gelation ability

The formulations' Gelation time ranges from 82 sec to 120 sec. As the polymer concentration increased, gelling ability improved correspondingly [30, 31].

In *vitro* % drug release of in situ gel batches:

The drug release curves presented in Figure 3 illustrate the cumulative drug release for 7 hours. The summarized data indicates that Formulation 10 achieved 94.66±0.57% drug release by 7 hours, demonstrating a rapid-release profile suitable for immediate therapeutic effects. The kinetics of drug release were determined from the optimized formulation.

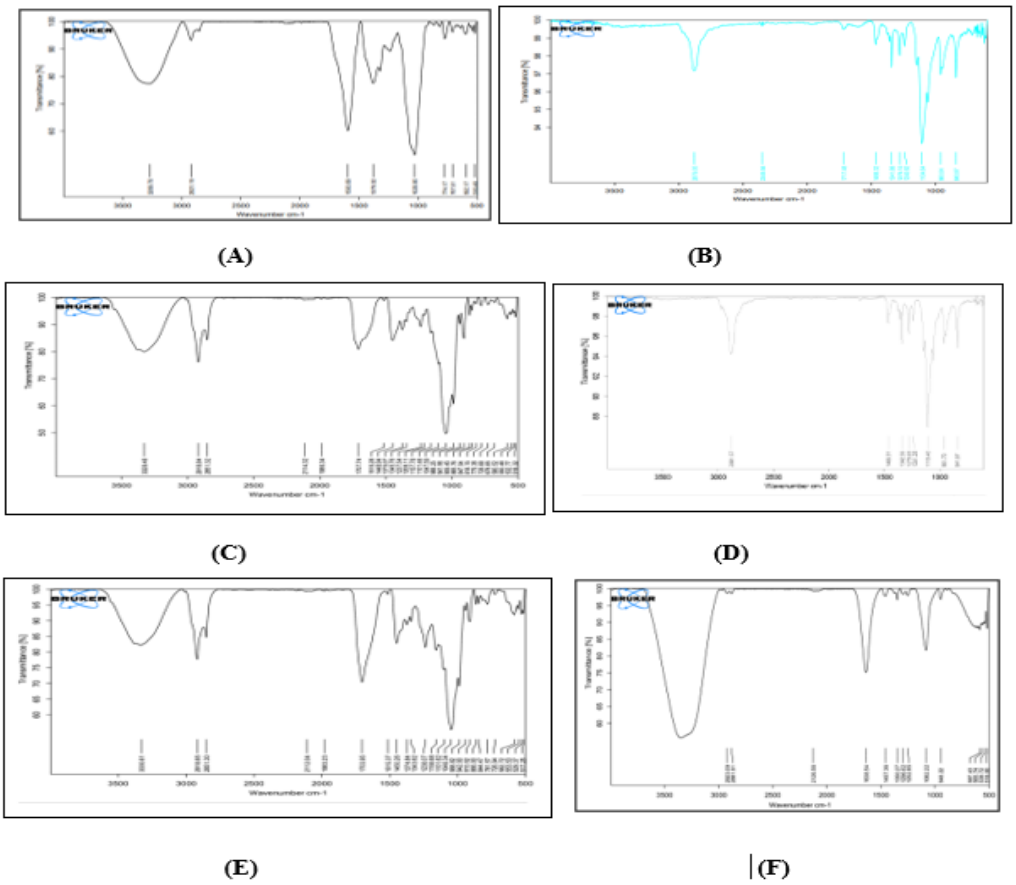

Figure 2: FTIR peaks of A: Ethanolic fruit extract of *Luffa acutangula* B: Extract+Poloxamer 407 C: Extract+Poloxamer 188 D: HPMC K40 E:: Extract+Carbopol 940 F: In situ gel

Table 3 pH, Gelation temperature, Gel strength, Gelling ability and Gelling time (sec) of in situ gels

Batch code	pH	Gelation temperature (ºC)	Gel strength	Gelling ability	Gelling time (sec)
F1	6.6	32.35±0.890	7.17±0.896	+	91.32±0.756
F2	6.8	48.90±0.102	5.23±1.02	++	100.87±0.879
F3	6.7	34.98±0.389	7.35±0.574	+	87.56±0.867
F4	6.9	37.75±0.256	6.64±0.675	+	120.45±0.468
F5	6.8	44.29±1.029	5.76±0.387	++	90.47±0.365
F6	6.9	36.35±0.835	6.08±0.756	++	92.09±0.489
F7	6.7	36.95±1.274	7.75±0.645	+	98.75±0.874
F8	6.8	38.75±0.457	5.08±0.145	++	89.75±0.397
F9	6.9	34.62±0.573	4.76±0.835	+++	85.68±0.460
F10	6.7	36.35±0.890	6.34±0.675	++	120.45±0.485
F11	6.9	30.90±0.102	6.96±0.387	++	94.47±0.354
F12	6.6	43.98±0.389	5.06±0.387	+++	82.09±0.48
F13	6.8	38.75±0.256	6.58±0.756	++	98.75±0.864

F14	6.7	28.29±1.029	6.75±0.645	+++	89.75±0.397
F15	6.7	35.38±0.835	5.45±0.145	++	85.18±0.47
F16	6.9	48.47±1.274	4.16±0.835	+	100.45±0.468

+ Phase transition after 60 sec and collapse of gel structure within 5 min, ++ Phase transition after 60 sec and collapse of gel structure within 10 min, +++ Phase transition within 60 sec, and gel remained stable for more than 30 min.

Table 4: % Drug content, viscosity of gel phase, and *In vitro* % drug release of in situ gel batches

Sr. No.	Batch code	% drug content	Viscosity of gel phase in (cps)	Invitro drug release %
1	F1	97.61±0.146	31600	93.73±0.56
2	F2	95.06±0.375	33668	80.37±0.10
3	F3	96.50±0.398	37089	92.75±1.09
4	F4	94.61±0.876	36968	90.94±1.16
5	F5	98.78±0.378	31786	82.59±0. 75
6	F6	90.17±0.678	30840	88.89±1.34
7	F7	99.33±0.468	35876	86.31±0.04
8	F8	99.06±0.647	31472	84.75±1.67
9	F9	99.89±0.735	30680	89.41±0.03
10	F10	99.33±0.468	34089	94.66±0.57
11	F11	94.06±0.370	36868	92.24±1.14
12	F12	95.50±0.338	31786	89.65±0.15
13	F13	97.61±0.866	32850	89.14±1.96
14	F14	98.18±0.372	35876	85.15±0.67
15	F15	90.17±0.675	35678	91.13±0.12
16	F16	96.61±0.116	332680	82.11±0.17

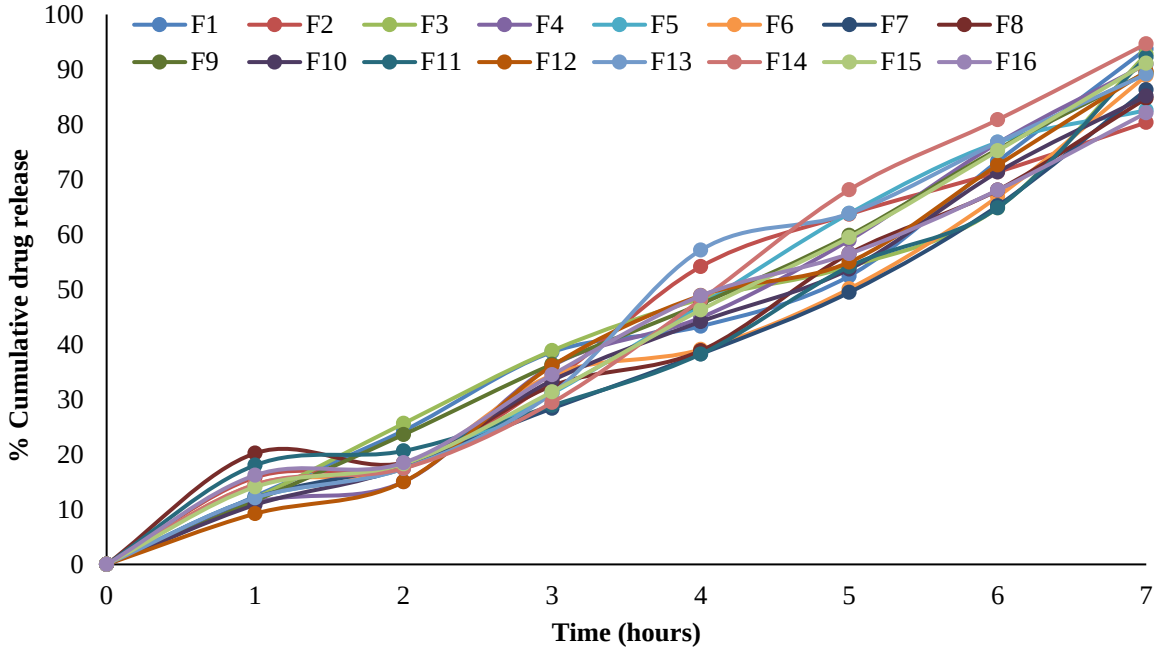

Figure 3: Dissolution plot of % cumulative drug release of *Luffa acutangula* in situ gels

Kinetic modeling of in *vitro* release study

Drug release from the optimized formulation follows the Higuchi release model, and according to Korsemeyer Peppa's model, the value of n=1.1114 indicates super case -II transport.

Full factorial design

Formulations were prepared according to the trial runs suggested by Design Expert Software Version 13. The experimental data were analyzed using ANOVA [16].

Table 5: Kinetic analysis

S. No.	Particulars	R^2
1	Zero order model	0.9764
2	First Order Model	0.7318
3	Higuchi Model	0.9903
4	Hixson Crowell Model	0.8421

Effect of formulation variables

Gelation temperature

The quadratic model had a significant F- value of 11.34 p-value < 0.0007 with a non-significant lack of fit 0.0529. The following quadratic model fitting equation describes the effect of the concentration of polymers on gelation temperature.

$Y_1=+36.54-6.17X_1-1.60X_2+0.3376X_1X_2+0.7498X_1^2+0.6978X_2^2$

The gelation temperature decreased with an increased concentration of P407 and increased with an increased concentration of P188.

% Drug release

The following quadratic model fitting equation describes drug release:

$Y_2= +87.69+4.00X_1+1.70X_2-2.03X_1X_2-0.6431X_1^2+2.93X_2^2$

The factorial design shows the significant quadratic model with an F- value of 16.42 p-value < 0.0002 with a nonsignificant lack of fit of 0.3488. Contour plots and three-dimensional surface response plots of the experimental results were drawn using design expert software 13.

Table 6: Summary of regression coefficients and ANOVA for response surface quadratic model

Response	F-value	p-value	Lack of fit	
			F- value	P- value
Gelation Temperature (°C)	11.34	0.0007	4.23	0.0529
% drug release	16.42	0.0002	1.30	0.3488

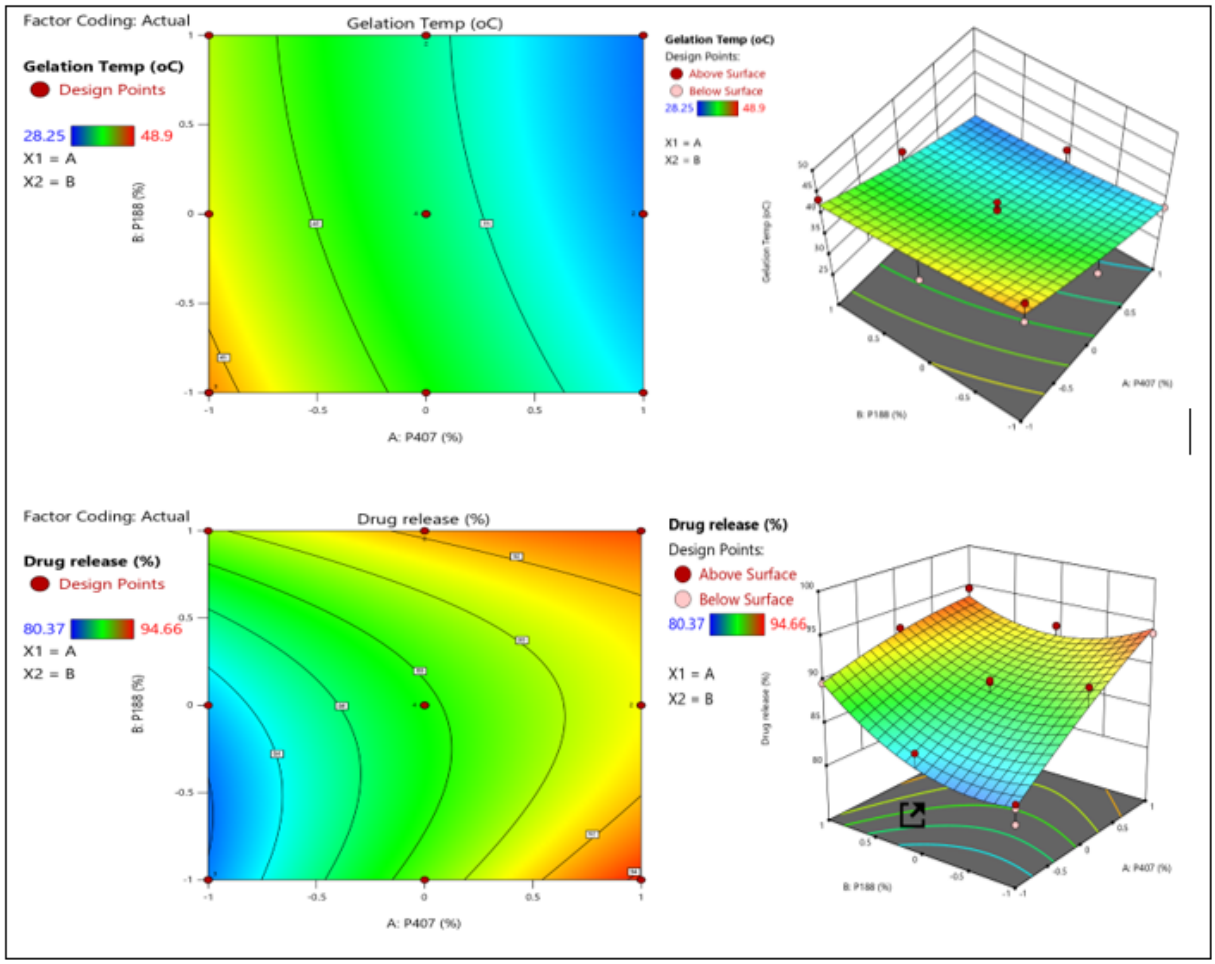

Figure 4: Contour plots and surface response plots of gelation temperature and drug release (response variable) and concentration of poloxamer 407 and poloxamer 188 as independent variables

Table 7: Characteristics of mucoadhesive in situ gels

Batch code	Gelation temperature (°C)	Gel Strength	Cumulative drug release	Mucoadhesive strength (dyne/cm^2)
F10HP1	36.05±0.375	5.66±0.57	90. 44±0.37	5748
F10HP2	36.45±0.102	4.36±0.471	91.37±0.84	6992
F10HP3	30.98±0.896	4.57±0.467	83.75±0.62	5998
F10CP1	33.02±0.368	6.33±0.57	82.64±0. 48	5717
F10CP2	29.67±0.896	7.0±1.0	81.59±0.34	6338
F10CP3	28.09±0.367	7.6±0.57	80.89±0. 27	6660

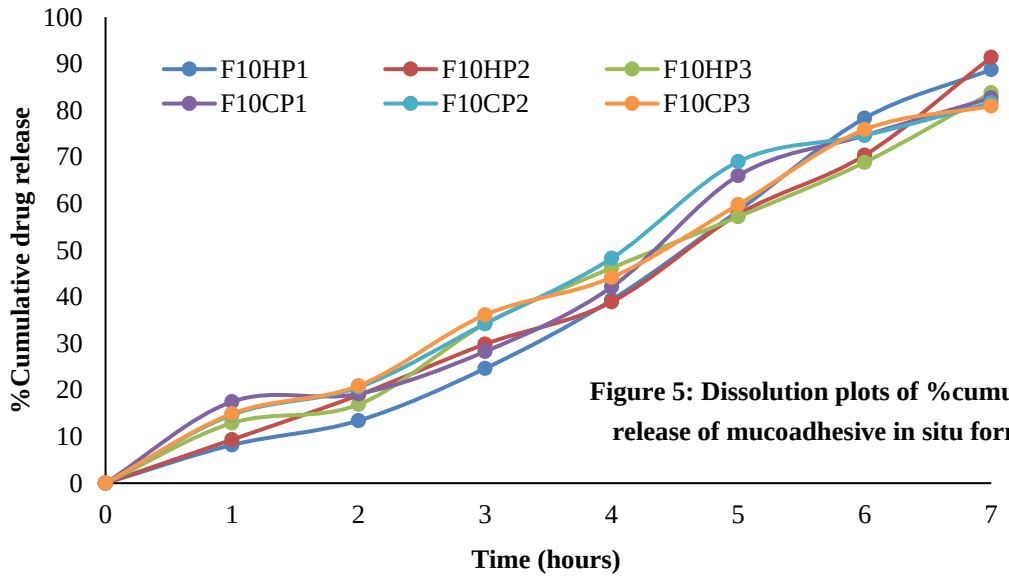

Figure 5: Dissolution plots of %cumulative drug release of mucoadhesive in situ formulations

Characteristics of mucoadhesive in situ gel

HPMC K40 and Carbopol 940 improve the gel's ability to adhere to mucosal surfaces. The lower concentrations of Poloxamer 188 and higher concentrations of Poloxamer 407 can create a gel with good mucoadhesive properties and optimal viscosity for easy application. The *Luffa acutangula* rectal in situ gel's strong mucoadhesive properties help minimize the drainage of the drug from the rectal area. The therapeutic efficacy of rectal gels is significantly influenced by their mucoadhesive properties and drug release profiles. Ensuring prolonged retention, targeted delivery, improved absorption, and controlled release provides effective treatment for various conditions while enhancing patient compliance and minimizing side effects. The gels promise that the active ingredients remain in contact with the absorption sites for an extended period, thereby improving bioavailability and overall therapeutic efficacy [43, 44].

In vivo animal study

Effect of extract of *Luffa acutangula* on % change in body weight

Intrarectal administration of AA (acetic acid) exhibited a significant decrease ($p<0.001$) in % Body weight in the disease control group when compared with the normal control group. The Mesalamine (300mg/kg) produced a significant increase ($p<0.001$) in % Body weight in standard control (SC) rats when compared with disease control (DC) rats. Intrarectal Administration of *Luffa acutangula* extracts (1ml/kg) significantly increased ($p<0.01$) in % body weight in treatment control (TC) when compared with DC rats [50].

Effect of extract of *Luffa acutangula* on Disease Activity Index (DAI)

Intrarectal administration of AA exhibited a significant increase ($p<0.0001$) in the Disease Activity Index in DC rats compared to NC rats. Mesalamine (300mg/kg) produced a significant decrease ($p<0.001$) in standard control as compared with the disease control group. Intrarectal administration of *Luffa acutangula* extracts (1ml/kg) significantly decreases ($p<0.001$) in TC when compared with DC rats [55].

Effect of extract of *Luffa acutangula* on proinflammatory cytokine Interleukin 6 concentration

Intrarectal administration of acetic acid showed a significant increase ($p<0.001$) in Interleukin levels in DC rats compared to the NC group. Intrarectal administration of Mesalamine (300mg/kg) produced a significant decrease ($p<0.0001$) in Interleukin levels in SC rats when compared with the DC group. Intrarectal administration of *Luffa acutangula* extracts (1ml/kg) significantly decreases ($p<0.001$) Interleukin levels in TC when compared with DC rats [54]. Studies suggest that extracts from *Luffa acutangula* will reduce the levels of pro-inflammatory cytokines (IL-6) in the body and play a significant role in inflammatory processes, indicating its potential effectiveness in inflammatory bowel diseases (IBD), including UC [56].

Histopathological evaluation

Histopathological examination indicates damage to the rectum with bleeding in disease control as compared to normal control. No or less bleeding with wounds in standard control and treatment control can be seen compared to disease control.

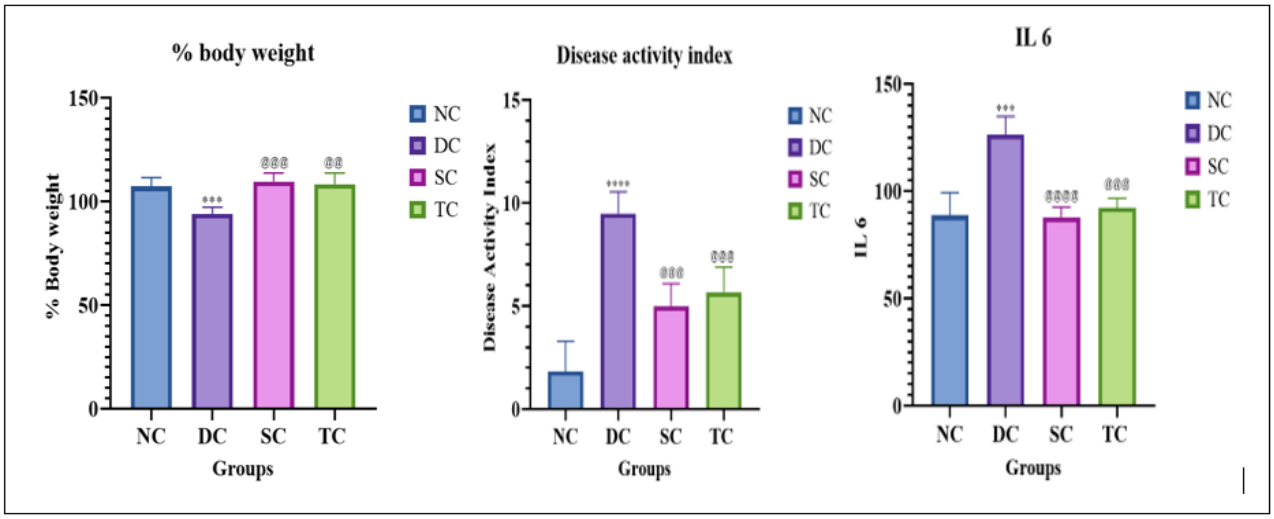

Figure 6: Effect of extract of *Luffa acutangula* on % change in body weight, Disease activity index, and proinflammatory cytokine Interleukin 6 concentration in Rats

***p<0.001 when DC compared with NC rats; $^{@@}$p<0.01 and $^{@@@}$p<0.001 when Treatment groups compared with disease control; NC = Normal control, DC = Disease control, SC = Standard control, TC = treatment control; Results are expressed as mean ± SEM (n=6); Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using Graph Pad Prism.

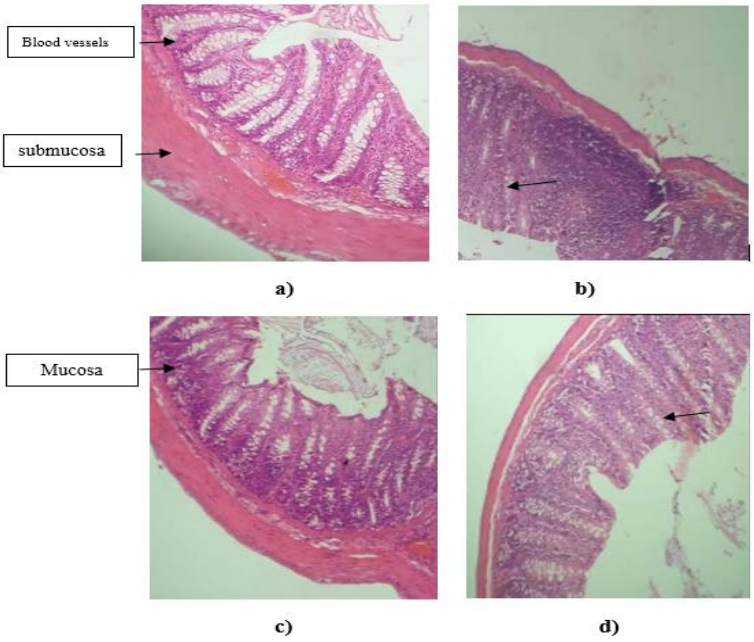

Figure 7: Histopathology of the mucosal layer of the intestinal region in acetic acid-induced ulcerative colitis groups as a) Group (I) - Normal control (b) Group (II) – Acetic acid control (c) Group (III) – *luffa acutangula* extract containing In situ gel treated (d) Group (IV) – Mesalamine treated

CONCLUSION

The thermosensitive rectal in situ gel was prepared using ethanolic fruit extract of *Luffa acutangula* with a cold method. The design expert software optimized formulation F10, which combined P407 (15%) and P188 (3%), showed suitable gelation temperature, gel strength, and gelling ability to enhance retention at the rectum. The dissolution study gave 94.66±0.57% cumulative drug release in thermosensitive gel and 91.37±0.84 % cumulative drug release in mucoadhesive gel. Drug release from optimized formulation followed the Higuchi release model. The mucoadhesive in situ gel of *luffa* extract F10HP2, which includes poloxamer 407, poloxamer 188, and HPMC K4M, was found to be much more effective than Carbopol-containing gel. *Luffa acutangula* gel can stop or slow down the progression of ulcerative colitis. The formulation (F10HP2) was designed successfully to provide safe and stable drug delivery for colitis treatment.

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NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Avish D. Maru designed the research study and interpreted the associated experimental data, while Swati P. Barangule conducted laboratory work, analyzed the data, interpreted the results, and wrote the manuscript.

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