Formulation Design and Ex Vivo evaluation of carbopol -Incorporated Mucoadhesive Thermoreversible gels of Sucralfate for rectal drug delivery

ABSTRACT

SAMPLE ABSTRACT:

Aim: The present research is focused on preparation of Thermoreversible gels and screening in vivo anti-inflammatory activity using croton oil induced hemorrhoid model.

Study design: The animals will be divided into four groups, n=8. Hemorrhoids were induced in all groups except control group by applying croton oil preparation, After overnight fast, hemorrhoids were induced by inserting sterile cotton swabs soaked in croton oil preparation into the anus of all group animals except normal control group.

Place and Duration of Study: Depart of Pharmacology, Sree Vidyanikethan College of Pharmacy, Tirupathi. between October 2021 to December 2021

Methodology: Experimental runs for optimization of formulation with the help of 2^3 factorial designs were designed [10]. Design Expert program 6.0.6 (Stat-Ease Inc. Minneapolis, MN) was used to create formulations. Formulations were optimized using full factorial design. *In situ* rectal gel was prepared by the cold method [11]. As shown in Table 1, P188 was dissolved in DDW (de-ionized distilled water) including drug (1% w/v) at room temperature, and it was stored at 4°C for complete solubilization of P188. Carbopol solubilized in DDW, HPMC K4M and other mucoadhesive polymers were then slowly added to the prepared solution with continuous agitation at 4°C and was kept for 24 h before their use. The composition of developed gel formulations is summarized in Table 1.

Results: Results In the first set of experiment, the application of croton oil to recto anal portion of rats induced significant increase in the RAC and serum level of pro inflammatory cytokines such as IL-6, and TNF-α. it also showed a significant increase in recto anal portion compared to normal control animals. However, the treatment with Sucralfate, Phenylephrine containing Thermoreversible gels has maintained the RAC and IL-6 levels near to normal control group. These findings were further supported by histopathological examination wherein the animals treated with optimized formulation showed significant reversal in the severity of inflammation, vasodilatation in the recto anal portion, and presence of inflammatory cells.

Conclusion: The findings of the present study suggest that the improved experimental model described in the present work has advantage. as this model has a provision for quantifying the inflammatory exudates in the induced experimental hemorrhoids. Also, it was found that, the optimized formulation ameliorated the croton oil-induced hemorrhoids in rats.

Keywords: *Rectal, Drug release, Mucoadhesive, Thermoreversible gel, Sucralfate*

1. INTRODUCTION

In the early 1980s, the concept of mucosal adhesion or mucoadhesive was introduced into the controlled drug delivery area, and it is now a major part of a novel drug delivery system. Some of the potential sites for attachment of any mucoadhesive system make use of the property of adhesion of certain water-soluble polymers to the buccal cavity, nasal cavity, eyes, vagina, rectal area, sublingual route, and gastrointestinal area. [1].

The use of a mucoadhesive system is required to ensure close and extended contact between the formulation and the rectal mucosa, allowing for a longer absorption time. When exposed to heat, thermoreversibility is a feature of certain substances that allows them to be reversed. When cooled, such substances form a gel, but when heated, they revert to a viscous fluid condition. [2].

Rectal administration employs the rectum as a route of administration for medication and other fluids, which are absorbed through the rectum blood vessels. A drug delivered rectally has a faster beginning of effect. [3]. When a local or systemic effect is desired, the rectal route is a viable, non-invasive alternate route of administration. The rectum provides a somewhat stable environment for drug delivery, allowing for a constant steady-state drug concentration in plasma and avoiding gastrointestinal absorption issues and hepatic first-pass metabolism to some extent. [4]. As a result, the current study was conducted to develop and test mucoadhesive thermoreversible gels of sucralfate for rectal drug delivery, as well as to improve the drug's bioavailability when compared to the oral route..

Hemorrhoids are swollen and dilated blood vessels that occur in the lower rectum and anus. [5]. Internal haemorrhoids develop in the area of the rectum above the pectinate line, which separates the upper two-thirds of the anal canal from the bottom one-third. Internal haemorrhoids are coated in a mucosa lining that is impervious to touch, discomfort, straining, or temperature changes. [6].

Phenylephrine is a decongestant that can also be used to enlarge the pupil, raise blood pressure, and treat haemorrhoids. Phenylephrine can be applied topically to treat haemorrhoids. Phenylephrine causes smooth muscle constriction and is frequently

prescribed for the treatment of haemorrhoids, ostensibly to reduce pain and enlarged veins. Because veins contain less vascular smooth muscle in their walls than arteries, they are less likely to rupture, [7].

2. MATERIAL AND METHODS

Materials

Sucralfate was obtained as a gift sample from Strides Arcolab, Bangalore (Purity 97%). Poloxamer 188 (P188) and Carbopol 934P (C934P) were purchased from Sigma-Aldrich. HPMC K4M was gifted by Colorcon Asia Pvt. Ltd., Goa India. All other chemicals and reagents used in the study were of analytical grade.

Preparation of formulations

Experimental runs for optimization of formulation with the help of 2^3 factorial designs were designed [10]. Design Expert program 6.0.6 (Stat-Ease Inc. Minneapolis, MN) was used to create formulations. Formulations were optimized using full factorial design. In situ rectal gel was prepared by the cold method [11]. As shown in Table 1, P188 was dissolved in DDW (de-ionized distilled water) including drug (1% w/v) at room temperature, and it was stored at 4°C for complete solubilization of P188. Carbopol solubilized in DDW, HPMC K4M and other Mucoadhesive polymers were then slowly added to the prepared solution with continuous agitation at 4°C and was kept for 24 h before their use.

Permeation studies through rectal mucosa

Permeation studies were carried out using rectal mucosa. The modified Franz diffusion cell assembly was used. Throughout the study the whole assembly was kept at $37^oC \pm 2^oC$. The medium used in acceptor compartment was 6.4 buffer solution, which was continuously stirring by placing on a magnetic stirrer. The samples were withdrawn at predetermined, regular time intervals and an equal amount of fresh medium was replaced. Amount of drug in the withdrawn samples was determined after suitable dilutions

Experiment design

Wistar rats were divided into four groups, Group I served as normal control and received only distilled water. Group II served as positive control and received 6% croton oil (10 ml). Group III was administered with Standard Formulation and 6% croton oil (10 ml). Group IV was administered with Optimized Formulation and 6% croton oil (10 ml). After overnight fasting, Evans blue (30 mg/kg i.v.) was injected in tail of animals of all the groups. After 30 min, hemorrhoids were induced in all groups except normal control group by applying croton-oil preparation (deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether in the ratio of 1: 4: 5: 10). Sterile cotton swab (4mm in diameter) soaked in cotton oil

preparation (100 ml) was inserted into the anus (about 22mm diameter) and kept for 10 s. development of edema was observed within 7 to 8h of induction of croton oil. After 24 h of induction, relevant treatment was given to all the groups for five days. On fifth day, 1 h after the treatment, blood was collected from the retro-orbital sinus. Inflammatory cytokines such as PG, TNF- α and IL-6 was estimated in blood by using Elisa Microplate Reader (Erba Lisa Scan II, Mannheim). All animals were euthanized by exsanguinations under deep isoflurane anesthesia and rectoanal tissue (20mm in length) was isolated and weighed. The RAC was calculated using the formula

Recto anal coefficient (RAC)

$$\text{Rectoanal coefficient} = \frac{\text{Weight of rectoanal tissue (mg)}}{\text{Body weight (g)}}$$

Severity Score

The isolated recto anal tissue was visually observed and scored based on the severity; the findings showed a severity score of 0.12 ± 0.35 in normal control group and 1.50 ± 0.22 (P < 0.01) in the positive control group, which was statistically significant compared to normal control, Interestingly, the treatment with standard formulation ; p<0.01, treatment with Optimized formulation; p<0.05, For histological examination, same tissue was examined for severity score and rectoanal-coefficient by fixing the tissue in 10% neutral buffered formalin[17].

Biochemical Estimations

Hematological examination Hemoglobin (Hb), White blood cell (WBC), platelet count (PLT), were examined through fully automatic cell counter (Mindray Vector, Model No.BC-2300).

Cytokines assays

Cytokines parameters such as tumor necrosis factor-α (TNFα), Interlukin-6 (IL-6) were assayed in the serum samples by ELISA Reader (Merck, Serial No. 21041098, MIOS-Jounior)[18].

Histopathological study

Histological observation of the recto anal tissue was made to note the appearance of inflammatory cells, congestion, hemorrhage, vasodilatation, and medium to high degrees of necrosis[20]

Stability studies

The property of formulations to maintain its physical, chemical, microbial, therapeutic and toxic standards in storage container in its shelf-life is called as pharmaceutical stability. Stability testing permits recommended storage settings, re-test intervals, and shelf-lives by revealing how properties of a formula ingredient changes over time when exposed to different temperature, humidity, and light conditions.[10]

In most circumstances, observing the pace at which a product declines at ambient temperature takes more time. To avoid this unfavourable damage, faster stability investigations are proposed.

ICH stipulates duration for investigation as well as the circumstances of storage.

- **Long-Term Analysis**: 25°C ± 2°C at 60% RH ± 5% for 12 Months
- **Accelerated Analysis:** 40°C ±2°C at 75% RH ± 5% for 6 Months

Procedure:

The purpose of the research was to see how temperature affected the optimal formulation \ For the formulation F5, stability tests were conducted for 3 months at 40°C ± 2°C with 75 RH 5%. Small amount of formulation in cillin bottles was preserved in desiccator with a concentrated sodium chloride to maintain a relative humidity of 75%. After one, two, and three months, samples were taken from the desiccator, which was placed in a hot air oven set 40°C ± 2°C. [21]

3. RESULTS AND DISCUSSION

Ex Vivo permeations

The % cumulative drug release was calculated against time and graph was plotted, whole experiment was performed in the triplicate. Ex-vivo diffusion profile was studied by freshly excised and treated sheep nasal mucosa. Bath temperature was maintained at 37±0.5°C throughout the experiment. Drug release profiles were showed in Figure 1. From the results obtained, it was concluded that as the concentration of polymers increased, there was a decrease in the drug release rate. [14]

Table no1: Sucralfate- Permeation Studies

S. No	Time	Formulation Code							
		F1	F2	F3	F4	F5	F6	F7	F8
1	40	30.45	50.15	28.15	27.35	24.15	30.15	29.15	26.15
2	80	49.41	59.23	35.13	38.23	36.27	37.37	35.56	36.89
3	120	62.23	62.83	45.76	47.16	49.76	46.16	48.77	47.73
4	160	73.41	78.51	77.51	75.51	79.87	72.87	81.47	79.87
5	200	87.53	88.61	86.61	85.23	98.93	83.83	97.23	82.83

Figure.1. Ex Vivo Permeation Studies

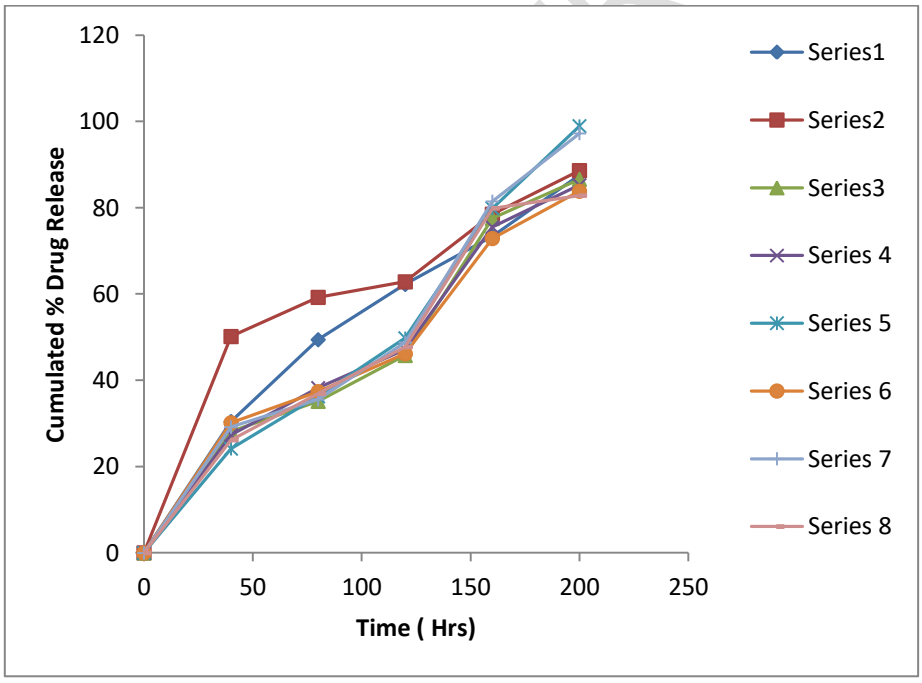


Table No 2: Recto anal coefficient (RAC)

Parameters	Group I	Group II	Group III	Group IV

	(Control)	(Croton oil)	(Stand Formulation)	(Optimum Formulation)
Recto-anal weight (mg)	181.0 ± 24.92	570.1 ± 41.61^{+++}	248.0 ± 21.13^{+++}	211.0 ± 15.32^{+++}
RAC	0.91 ± 0.32	2.43 ± 0.40^{+++}	1.16 ± 0.16^{+++}	1.30 ± 0.5^{+++}

All data are the means and standard deviations of eight animals in each group. The statistical significance of the control group (GI) vs. hemorrhoids induced group (GII) and hemorrhoids induced group + Stand Formulation (GIII) hemorrhoids induced group + Optimized Formulation treated group was determined (GIV)

+++p< 0.0001: High Significant, ++p<0.001: Significant, +p<0.05: Less Significant and Ns p >0.05: Insignificant

Severity Score

Physical examination of isolated Recto anal tissue was done and based on severity it was scored. results revealed a severity score of 0.14 ± 0.25 in the normal control group and 1.60 ± 0.32 (P < 0.01) in the positive control group, which was probability significant when compared to normal control. And treatment with standard formulation shown; p<0.01 and treatment with Optimized formulation; p<0.05.[12]

Biochemical Estimations

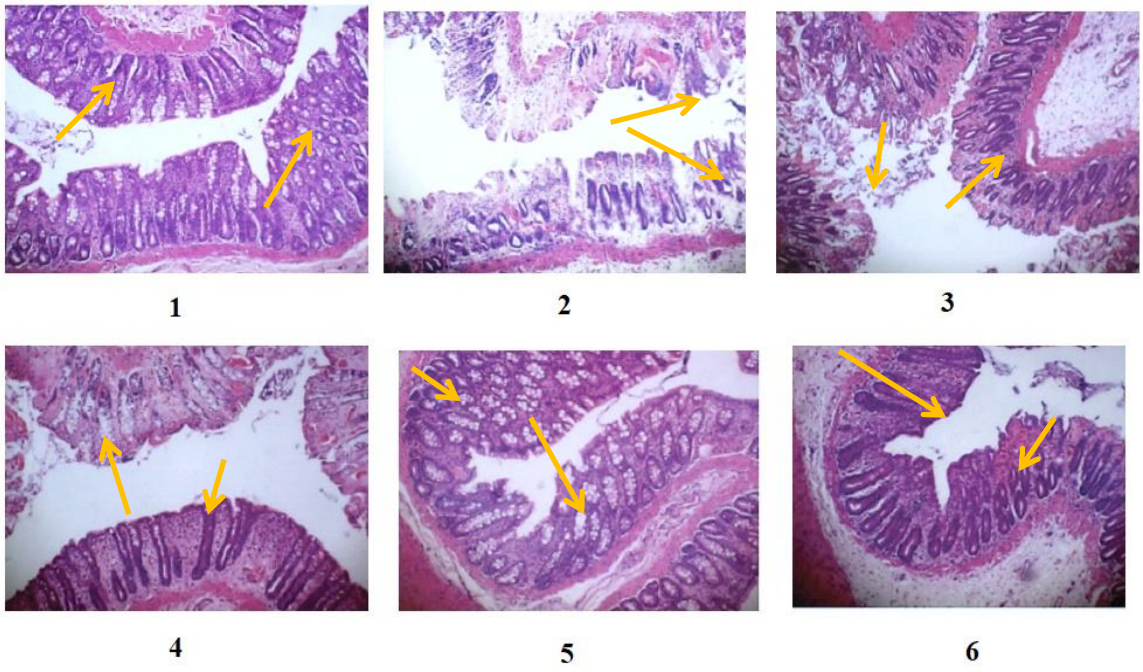
Table no 3: Hematological examination.

	Control	6% Croton Oil	Standard Formulation (for 5 days)	Optimized Formulation (for 5 days)
Hemoglobin (Hb)	15.80 ± 0.64	11.58 ± 1.58***	14.73 ± 0.39***	15.03 ± 0.19***
White blood cell (WBC)	11.4 ± 0.65	17.30 ± 3.63***	13.45 ± 1.85**	12.12 ± 2.45**
Platelet count (PLT)	604.75 ± 62.89	192.25 ± 19.22***	525.25 ± 56.72***	610.25 ± 16.32***

Table no 4: Cytokines assays

	Control	6% Croton Oil	Standard Formulation (for 5 days)	Optimized Formulation (for 5 days)
Tumor necrosis factor-α	4.70 ± 0.329	15.88 ± 0.186	6.71 ± 0.037	5.12 ± 0.046
Interlukin-6	2.15 ± 0.141	6.38 ± 0.137	3.91 ± 0.072	3.01 ± 0.021

Figure No 2: Histopathological study



1- The normal control group had a normal appearance and structure.

2- In untreated positive control group, there is marked to severe inflammation, haemorrhage, congestion, degeneration, dilatation of blood vessels, and necrosis.

3 & 4 - The rat rectal region treated with standard Sucralfate gel demonstrating minimal inflammation, congestion, haemorrhage, blood vessel dilatation, degeneration, and necrosis;

5 - The rat rectal region treated with Sucralfate thermoreversible gel (F5) showing near normal (H&E × 200)

6 - The rat rectal region treated with Phenylephrine thermoreversible gel (F5) apper near normal (H&E × 200)

Stability studies

Formulations were analyzed for various evaluation parameters after exposure to stability conditions (40ºC ±2ºC at 75% RH ±5% RH)[11]

Table no 5: Stability studies of optimized formulation Sucralfate

	Initials	1st Month	2nd Month	3rd Month
Clarity	+++	+++	+++	+++
Gelation Temperature (F5)	36.2±0.11	36.18± 0.30	36.15± 0.23	36.11± 0.9
pH	6.8±0.34	6.57 ±0.13	6.6 ±0.07	6.52± 0.42
Drug Content	97.3±0.07	97.16±0.22	97.1±0.32	97±0.28
Invitro Drug release	99.38±0.21	99.21±0.32	99.1±0.78	98.4±0.67

The studies revealed that there were no significant differences in the evaluated data from initial after stability studies of Clarity, pH, Gelation temperature, P^H drug content, and in-vitro drug permeation studies, and all of the values were found to be within acceptable limits after the stability studies at 40°C 2°C at 75 percent RH 5% for optimized formulations.

4. CONCLUSION

Form the prepared thermoreversible Mucoadhesive gels, Formulation F5 appears to have the greatest potential for producing thermoreversible rectal gels out of the eight formulations. Some of the assessment factors used to assess the accuracy of formulation ex-vivo permeation studies, formulation F5 was found to be the best for treating hemorrhoidal activity with a temperature-induced Rectal in situ gelling device.

CONSENT (WHERE EVER APPLICABLE)

"All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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