

Colon Targeting of Naringin for Cytoprotection against Ulcerative Colitis: In Vitro-In Vivo Study

Thanaa M. Borg^a, Elham A. Mohamed^a, Eman E. El Naggar^{a,*},
Ahmed R. El-Sheakh^b, Mohammed F. Hamed^c

^aDepartment of Pharmaceutics, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt

^bDepartment of Pharmacology, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt

^cDepartment of Pathology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, 33516, Egypt.

Abstract: Naringin has low oral bioavailability because of its degradation at the upper gut. Therefore, this study involved formulation of compression-coated tablets of naringin using mixtures of Eudragit L100-55 (EUD-L) and hydroxypropyl methylcellulose (HPMC) at two different respective weight ratios of 95:5 (F1) and 90:10 (F2). Evaluation of the tablets regarding drug content, physical characters, swelling and *in vitro* release has been performed. Kinetic analysis of release data was done. The effects of the selected tablet formulation on indomethacin-induced colitis in rabbits were investigated. The results showed that average values of drug content were 99.66 ± 2.18 and 104.00 ± 1.58 for F1 and F2, respectively. The prepared tablets showed good hardness (7.97-8.85 kg/cm²) and friability (less than 1%) that were within the pharmacopeal range. The increase in HPMC content to 10% of the coating weight (F2) resulted in higher swelling degree of the coated tablets. Therefore, more retarded release and colon targeting were obtained with higher HPMC content (F2) as clarified by percentage drug release of 17.80 ± 2.70 after 5 h. In accordance, this tablet formulation provided effective protection against indomethacin-induced colitis in rabbits as confirmed by normal mucosa of colon, significant ($p < 0.05$) decrease in levels of serum pANCA and colonic TNF- α .

Keywords: Compression coated Tablets, Colon targeting, Eudragit L100-55, Hydroxypropyl methylcellulose, Naringin, Ulcerative colitis.

I. Introduction

The oral route is commonly used to administer drugs because of better patient compliance, easier administration and relatively lower production cost than other routes [1, 2]. Site-specific delivery systems for targeting drugs to the colon have been widely investigated during the last decade. These drug delivery systems have been utilized to deliver drugs locally in the colon to treat diseases like Crohn's disease, ulcerative colitis, irritable bowel syndrome and constipation as well as to provide a systemic action of various drugs such as proteins, antiasthmatics, antihypertensives and antidiabetics [3, 4]. Treatment of ulcerative colitis with glucocorticosteroids and immunosuppressive drugs may cause serious side effects [5, 6]. Naringin exhibited several pharmacological activities such as anti-inflammatory, cardiovascular, hypolipidemic, antiatherosclerotic, antidiabetic, neuroprotective, hepatoprotective, and anticancer activities [7, 8]. Naringin has shown some gastroprotection against colitis in mice [9]. However, cleavage due to the harsh pH and enzymatic conditions of the upper gastrointestinal tract resulted in low and irregular absorption of this drug following oral administration [10, 11, 12].

Site-specific drug delivery systems for colon targeting should hinder the drug release and absorption in upper gut but allow drug release in the colon [13]. The local treatment of inflammatory bowel diseases, such as ulcerative colitis is highly challenging since it requires minimal release in the upper gastro-intestinal tract (GIT) and time-controlled release within the colon [14, 15]. Coating of tablets with Eudragit-L100-55 (EUD-L) can protect the active drug from gastric fluid and possibly the proximal part of the small intestine [16]. Addition of water-insoluble polymer as hydroxypropyl methylcellulose (HPMC) to EUD-S100 coats has been used for colonic delivery of diclofenac sodium [17].

When compared to the traditional liquid coating process, direct compression coating of tablets for colon targeting is more convenient particularly for large scale production as it is solvent free, less time-consuming and inexpensive [18].

In this study, directly compressed coated tablets of naringin were prepared using coating mixtures of EUD-L and HPMC. The prepared tablets were evaluated with respect to drug content, hardness, friability, swelling, and *in vitro* release in media with different pH values (1.2, 6.8, and 7.4) up to 8 h to select an optimized formula. As well, the protection of the selected tablet formulation against indomethacin-induced colitis in rabbits was investigated.

II. Materials and method

Naringin and hydroxypropyl methylcellulose (HPMC K100) were purchased from Sigma-Aldrich Co., St. Louis, USA. Eudragit L100-55 (EUD-L) was obtained from Evonic Rhom GmbH, Darmstat, Germany. Indomethacin was supplied by Kahira Pharmaceuticals & Chemical Industries Co., Cairo, Egypt. Rabbit anti-antineutrophilic perinuclear antibodies (pANCA) ELISA Kit was purchased from My biosource, USA. Avicel pH 101 was obtained from Eipico Pharmaceutical Chemicals Co., Cairo, Egypt. Magnesium stearate was purchased from Acros organics, New Jersey, USA. Talc was purchased from El-Nasr Pharmaceutical Chemicals Co., ADWIC, Cairo, Egypt. Other chemicals used were of fine analytical grade.

2.1. Preparation of naringin core and compression coated tablets

Passage of naringin through 200 μm sieve was followed by mixing with avicel pH 101 using a mortar and pestle and then lubrication with a 2:1 blend of talc (2% w/w) and magnesium stearate (1% w/w). An amount of 180 mg of the mixture was directly compressed (Erweka, Germany) to give a core tablet containing 125 mg naringin with hardness between 5 and 6 kg/cm^2 .

The core tablets were coated with EUD-L and HPMC at two weight ratios of 95:5 and 90:10, respectively, maintaining the core: coat ratio at 1:2, Table 1. The polymers were passed through 200 μm sieve, mixed using a mortar and pestle for 10 min and then lubricated with talc (2% w/w) and magnesium stearate (1% w/w). A quantity of 180 mg of the polymers mixture was put into the die, the tablet core was placed at the center and finally another weight of 180 mg of the polymers mixture was added to be directly compressed (Erweka, Germany). The tablet hardness range was 8-10 kg/cm^2 .

Table (1): Composition of tablet coats

| Formula code | Eudragit L100-55 (EUD-L) | Hydroxypropyl methylcellulose K100 (HPMC) | Magnesium stearate | Talc |
|--------------|--------------------------|---|--------------------|------|
| F1 | 331.74 | 17.46 | 3.6 | 7.2 |
| F2 | 314.28 | 34.92 | 3.6 | 7.2 |

2.2. Characterization of naringin tablets

Physical characters of the tablets were studied according to procedures reported in US pharmacopoeia [19]. The drug content in the tablets was determined in triplicate. An amount of the ground ten tablets equivalent to 125 mg of naringin was mixed with methanol, sonicated then the final volume was adjusted with phosphate buffer (PB) pH 7.4 to 500 mL. The sonicated mixture was filtered and diluted. Spectrophotometric analysis (UV/VIS spectrophotometer, Jasco, Japan) was done for drug content at 282 nm.

2.3. Swelling studies

Each tablet was individually weighed (W_1) and placed into a beaker containing 200 mL of 0.1N HCl kept at $37 \pm 0.5^\circ\text{C}$. At time intervals of 30, 60 and 120 min, the tablet was reweighed (W_2) after a careful removal of the medium on the surface. The same process was followed in PB pH 6.8 and 7.4 using the same tablet. The mean weights of tablets were determined after repeating the process twice to calculate the percent swelling according to the following relation [2, 20]:

$$\text{Swelling index} = \frac{W_2 - W_1}{W_1} \times 100$$

2.4. In vitro drug release

The drug release from the coated tablets was studied using USP apparatus I (Dissolution Apparatus USP Standards, Scientific, DA-6D, Bombay, India). The dissolution medium (500 mL) was kept at $37 \pm 0.5^\circ\text{C}$ and stirred at 100 rpm. The release medium was 0.1 N HCl (pH 1.2) for 2 h, PB pH 6.8 for 3 h, and finally PB pH 7.4 [21, 22]. Samples were withdrawn at certain time intervals up to 8 h and replaced by fresh medium. The samples were filtered and diluted to be analyzed spectrophotometrically at 282 nm (UV/VIS spectrophotometer, Jasco, Japan).

2.5. Kinetic modeling of drug release data

In vitro release data were analyzed according to zero-order, first-order [23], diffusion-controlled release mechanism [24] and Korsmeyer-Peppas kinetic model [25]. The model with the highest correlation coefficient (r^2) was considered to describe the drug release.

2.6. In vivo evaluation of the optimized naringin tablets

2.6.1. Experimental animal protocol

Male New Zealand rabbits (≈ 2.5 kg) were used in this study in accordance with the ethical principles of the scientific committee of Faculty of Pharmacy, Mansoura University, Egypt for the use of experimental animals. As discussed later, the selected tablet formula for *in vivo* study was that coated with a combination of 90% EUD-L and 10% HPMC (F2), Table 1.

Eighteen rabbits were randomly divided into three groups, each consisted of six animals. The animals were fasted but allowed a free access to water for 24 h before the day of the experiment. Group I received saline. Group II was given 8 mg/kg indomethacin suspension in 1% sodium carboxymethylcellulose orally (untreated colitis) [26]. Group III was given 10% HPMC coated tablets (F2) for five days, then a single intragastric dose of 8 mg/kg indomethacin in 1% SCMC was given at the fifth day 2 h after coated tablet administration.

2.6.2. Measurement of serum pANCA

Twenty-four hours following indomethacin administration, collection and centrifugation of blood samples were done to separate sera to be stored at -80°C to be assayed for perinuclear antineutrophil cytoplasmic antibodies (pANCAs) using the kit of My bio source, USA.

2.6.3. Histopathological investigation of colon

At the end of the animal study, rabbits were sacrificed and pieces of colon were cleaned, washed with 0.9% (w/v) saline solution and kept in 10% (v/v) formalin in saline for histopathological investigation. Sections of $5\ \mu\text{m}$ were stained with hematoxylin and eosin.

2.6.4. Colonic levels of TNF- α

Staining was done using the histostain bulk kit—Invitrogen Lab-SA detection system. Deparaffinization was accomplished by EZ Prep solution. Citrate buffer of pH 6.0 was used for antigen retrieval. DAB inhibitor (3% H_2O_2 endogenous peroxidase) was blocked for 5 min at room temperature. Sections were incubated with anti-TNF- α antibody (Boster Biological Technology dilution 1/100) for 40 min at room temperature, then with the antibody of Universal HRP Multimer for 8 min at 37°C . Slides were treated with DAB + H_2O_2 substrate for 8 min, followed by hematoxylin and the bluing reagent counterstain at 37°C . Phosphate buffer saline (PBS) was used for washing. The staining intensity of positively stained cells was evaluated and immunostaining was scored [27]. Controls were prepared by staining without employment of primary antibody.

2.7. Statistical analysis

Statistical analysis of the results was accomplished applying One-way ANOVA and then Tukey–Kramer multiple comparisons test at a significance level of $p < 0.05$ employing Graphpad prism software (version 5.00; Graphpad software, San Diego, CA, USA).

III. Results and discussion

3.1. Characterization of the compression-coated tablets

The physical characteristics and drug content uniformity of the coated tablets are represented in Table 2. The tablets showed good weight uniformity. Average hardness values and friability (less than 1%) for the tested coated tablets were within the pharmacopeal limits [19]. The studied tablets exhibited uniform drug content within the pharmacopeal range of 90-110% [19].

Table (2): Drug content and physical characters of naringin tablets

| Formula code | Mean \pm SD | | | | | |
|--------------|------------------------|-----------------|------------------|--------------------------------|-----------------|-------------------|
| | Weight uniformity (mg) | Thickness (mm) | Diameter (mm) | Hardness (Kg/cm ²) | Friability (%) | Drug content (%) |
| F1 | 541.28 \pm 1.60 | 3.56 \pm 0.04 | 12.11 \pm 0.07 | 8.85 \pm 0.33 | 0.58 \pm 0.08 | 99.66 \pm 2.18 |
| F2 | 540.22 \pm 1.42 | 3.75 \pm 0.01 | 12.09 \pm 0.08 | 7.97 \pm 0.54 | 0.58 \pm 0.05 | 104.00 \pm 1.58 |

3.2. Swelling study

The swelling behavior of the coated tablets has been examined, Figure 1. The increase in the polymer concentration from 5% to 10% enhanced the coat swelling. At pH 1.2, high swelling percentages have been observed probably due to the rapid hydration and the high affinity to the test medium. At PB pH 6.8, the increase in the swelling rate continued being the maximum at 2.5 h. On the other hand, a sharp decrease in the swelling of coatings has been observed at PB pH 7.4 beyond the fifth hour that could suggest the erosion of these swollen matrices.

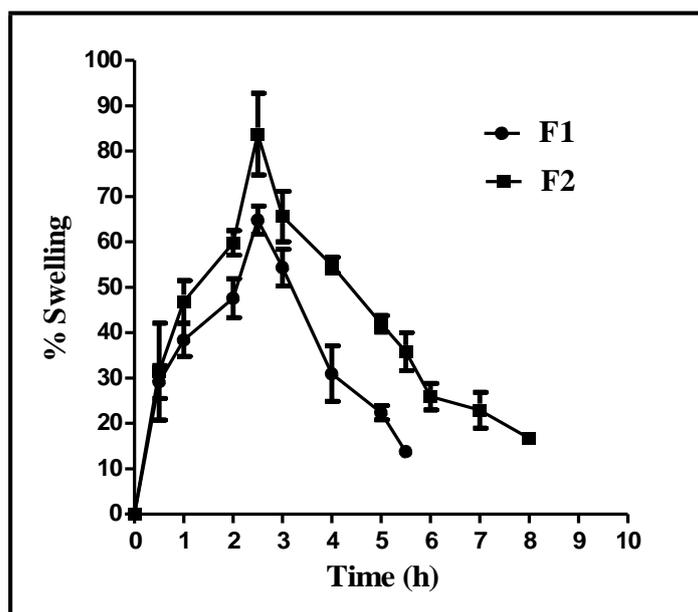


Figure 1: Swelling behavior of naringin tablets coated with 5% HPMC (F1) and 10% HPMC (F2) in 0.1N HCl for 2 h, PB pH 6.8 for 3 h, and PB pH 7.4 for 3 h.

3.3. In vitro release study

The results of *in vitro* release of naringin from tablets coated with Eud L-100-55 and HPMC are shown in Figure 2. Generally, the release was insignificant ($0.37\% \pm 0.13$) during the first two hours in 0.1N HCl. When HPMC concentration in the coat was 5% (F1), drug release continued to be low up to the first hour and half in PB pH 6.8, and then it increased reaching $86.42\% \pm 1.33$ after 5 h. On the other hand, coating containing 10% HPMC showed retarded release in PB pH 6.8 up to 5 h ($17.80\% \pm 2.69$). The cumulative % release less than 20% after 5 h has been used as indicative of colon targeting [28, 29]. As a result, tablets coated with 10% HPMC together with 90% EUD-L (F2) have been selected to investigate the protection activity of naringin against indomethacin-induced colitis in rabbits.

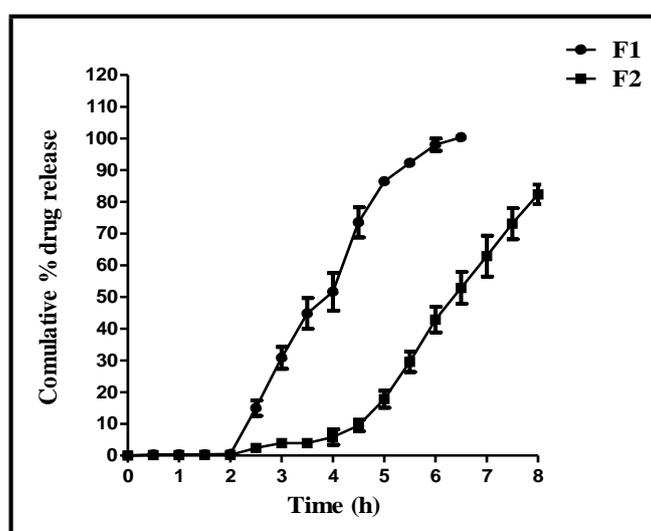


Figure 2: *In vitro* drug release of naringin tablets coated with 5% HPMC (F1) and 10% HPMC (F2) in 0.1N HCl for 2 h, PB pH 6.8 for 3 h, and PB pH 7.4 for 3 h.

3.4. Release kinetics

According to Table 3, the release mechanism fitted zero order kinetics. According to Korsmeyer-Peppas model, $n \leq 0.5$ may indicate Fickian diffusion and values of (n) between 0.5 and 1 correspond to non-Fickian release [25]. At pH 1.2, Fickian release mechanism characterized by diffusion described the drug release from both tablet formulations that may be explained by the high polymer swelling rates (Figure 1). While, non-Fickian release mechanism controlled by both diffusion and erosion can be assumed at pH 6.8 and 7.4 that could cause the higher release at these media.

Table 3: Kinetic modeling of drug release data

| Dissolution medium | Kinetic model | F1 | F2 |
|--------------------|---------------|----------------|----------------|
| | | r ² | r ² |
| 0.1 N HCL | Zero order | 0.918 | 0.923 |
| | First order | 0.918 | 0.927 |
| | Diffusion | 0.877 | 0.870 |
| | Korsmeyer | | |
| | n value | 0.384 | 0.332 |
| | mechanism | Fickian | Fickian |
| PB pH 6.8 | Zero order | 0.988 | 0.797 |
| | First order | 0.913 | 0.782 |
| | Diffusion | 0.968 | 0.707 |
| | Korsmeyer | | |
| | n value | 0.963 | 0.999 |
| | mechanism | Non Fickian | Non Fickian |
| BP pH 7.4 | Zero order | 0.988 | 0.997 |
| | First order | 0.937 | 0.976 |
| | Diffusion | 0.973 | 0.995 |
| | Korsmeyer | | |
| | n value | 0.954 | 0.569 |
| | mechanism | Non Fickian | Non Fickian |

3.5. Histopathological evaluation of rabbit colon

The colon of normal group (I) showed normal mucosa and submucosa, Figure 3A. Administration of indomethacin to group II resulted in ulceration of entire thickness of mucosa, Figure 3B. On the other hand, group III that received naringin tablet coated with 10% HPMC showed gastroprotection as indicated by normal mucosa with some infiltration in the submucosa, Figure 3C. These results may be explained on the basis that the release retardation imparted by tablets coated with 10% HPMC provided minimal gastric and intestinal absorption but allowed high drug release in the colon. The successful use of compression coated tablets of budesonide in treatment of ulcerative colitis has been documented [30].

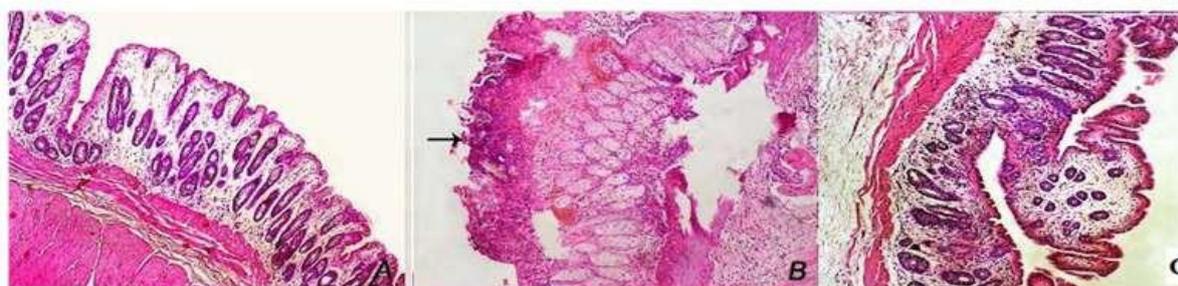


Figure 3. Photomicrographs of hematoxylin and eosin stained paraffin sections of rabbit colonic tissues (100x) showing normal mucosa and submucosa in normal group (I) (A), ulceration of superficial mucosa (arrow) in colitis untreated group (II) (B), normal mucosa with some infiltration in the submucosa in group (III) treated with 10% HPMC coated tablets (C).

3.6. Effect of naringin on pANCA serum level

In comparison with colitis untreated group (II), serum levels of pANCA were significantly ($p < 0.05$) lower in rabbits received naringin tablets coated with 10% HPMC (F2, group, III), Table 5. These results suggest such coated tablets as a promising colon targeting delivery system of naringin for protection against indomethacin-induced colitis in rabbits.

Table 5: Serum levels of pANCA in rabbits

| Treatment group | pANCA serum level (ng/mL) |
|---------------------------|----------------------------|
| II (Indomethacin; 8mg/kg) | 12.59± 0.69 |
| III (HPMC; F2) | 9.08± 0.40 # |

Data are represented as mean ± SEM (n = 6).

Significantly different compared to colitis untreated group (II) ($p < 0.05$)

3.7. Effect of naringin on colonic TNF- α levels:

The normal group (I) exhibited faint immunostaining indicating minimal immunoreactivity, Figure 4A. The colitis untreated group (II) showed strong immunoreactivity in the form of scattered fine brown granules, Figure 4B. On the other hand, rabbits received 10% HPMC coated tablets (group III) showed highly reduced TNF- α content than that of colitis untreated group (II), Figure 4C. The levels of colonic TNF- α were significantly ($p < 0.05$) high in colitis untreated group (II) compared to normal group (I), Table 6. While, the reduction in colonic TNF- α obtained on pretreatment with coated naringin tablets was significant ($p < 0.05$) relative to colitis untreated group (II).

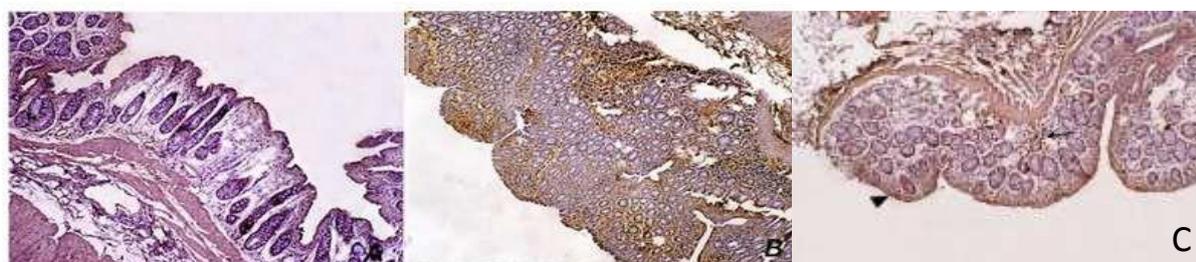


Figure 4. Immunohistochemical evaluation of TNF- α in all studied groups (100x) showing negative immunostain for TNF α in normal group (I) (A), strong positive immunoreactivity in submucosa in colitis untreated group II (B) less positive immunoreactivity limited to epithelial lining mucosa and endothelial lining blood vessels (arrow) in group III administered 10% HPMC coated tablets (C).

Table 6: Effect of naringin tablets on colonic TNF α scoring

| Treatment group | Scores of TNF- α) |
|----------------------------------|---------------------------|
| Group I (control; no drug) | 3.25± 0.87 |
| Group II (indomethacin; 8 mg/kg) | 93.25± 3.91* |
| Group III (10% HPMC; F2) | 28.75± 4.04*# |

Data are represented as mean ± SEM (n = 6).

* Significantly different compared to normal control group (I) ($p < 0.05$)

Significantly different compared to colitis untreated group (II) ($p < 0.05$)

IV. Conclusion

The low cumulative percent drug release ($< 20\%$) from compression coated tablets based on 10% HPMC and 90% EUD-L at gastric and intestinal pH indicated a successful colon targeting of naringin. This was confirmed by normal mucosa on histopathological examination and significant ($p < 0.05$) decrease in both serum levels of pANCA and colonic levels of TNF- α . The swelling of HPMC coated tablets at gastric and intestinal media (pH 1.2 and 6.8, respectively) could account for the delayed drug release at these media and expected colon targeting. Thus, compression coated tablets based on 10% HPMC and 90% EUD-L can be suggested as a promising colon targeting system of naringin for cytoprotection against ulcerative colitis.

Conflicts of interest

The authors declare no conflicts of interest.

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