
Research Article

Development and Gamma Scintigraphy Study of *Trigonella foenum-graecum* (Fenugreek) Polysaccharide-Based Colon Tablet

Nitin Sharma,^{1,3} Anjana Sharma,² Dhruv K. Nishad,¹ Kushagra Khanna,¹ Braj Gaurav Sharma,¹ Dipti Kakkar,¹ and Aseem Bhatnagar¹

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Abstract. The major concern with the use of some synthetic excipients is their safety towards biological tissues, hence influencing the reliability of products. With the aim to minimize dependency on highly toxic synthetic excipients, the present study was designed to deliver metronidazole (MNZ) into the colonic region for localized treatment of amoebiasis using natural polysaccharide-based drug delivery. Compression-coated tablets were prepared using water extractable natural polysaccharide from *Trigonella foenum-graecum* (FG). Physical properties of the tablets were evaluated and dissolution study was performed at pH 1.2, 6.8, and 7.4 with rat cecal material. Results indicate that all batches demonstrated pH-dependent drug release and prevented release into the stomach, allowing traces into the intestine and highest availability into the colon. A significant correlation ($r^2 = 0.975$) was found between the coating levels of extracted polysaccharide and lag time release of drug. Gamma scintigraphy images of *in vivo* study conducted on human volunteers showed a small intestinal transit time, i.e., 3–5 (4.2 ± 0.4) h and confirmed that the tablets reached the colon within 6–8 h. The present study revealed that the FG polysaccharide-based double compression tablets may be promising colon-specific drug carriers with reduced toxic effects of commonly used synthetic excipients.

KEY WORDS: *Trigonella foenum-graecum*; fenugreek; metronidazole; natural polysaccharide; colon tablet.

INTRODUCTION

Amoebiasis, a contagious disease of the tropical and subtropical region affects about 34–50 million people by infecting their gastrointestinal tract (GIT) and leading to 40–100 thousand deaths per year (1). It is caused by a micro aerophilic protozoan parasite *Entamoeba histolytica* which is responsible for chronic infections resulting in tissue degeneration with liver abscesses, amoeboma, and dysentery. Metronidazole (MNZ) is the most preferred choice of drugs for the treatment of amoebiasis which acts locally in the colonic region. Pharmacokinetic profile of MNZ has shown that this drug is absorbed rapidly from the upper part of GIT within 1 h of dose administration (500 mg). Drug administration through conventional dosage form provides availability of very little amount of the drug into the colonic region for local

action (2). Several side effects are also associated with this treatment due to rapid absorption of large amounts of the drug in the upper GIT despite its documented efficacy. Literature has reported MNZ-associated toxicity such as carcinogenicity and genotoxicity with long-term use of the drug (3). MNZ has been reported for colon delivery for its effective action against *Entamoeba histolytica* as the trophozoites reside in the lumen of the caecum and large intestine and adhere to the colonic mucus and epithelial layers (1). Therefore, it is logical to say that delaying absorption of drug until it reaches the colonic region would be a beneficial strategy.

Nowadays, researchers are moving towards the utilization of natural plant polysaccharide and gum as pharmaceutical excipients. Currently, various natural polysaccharides have been extracted from a number of plant sources. These natural polysaccharides have been found to be advantageous over synthetic polymers being used for the same purpose. These advantages include low cost, flexibility in obtaining desired release profile, biocompatibility, and/or biodegradability (4). Various plant polysaccharides like gaur gum (5), chitosan (6), alginate (7), and katira gum (8) have been investigated for effective drug delivery to the colonic region. These polysaccharides are also gaining attention specifically

¹ Department of Nuclear Medicine, Institute of Nuclear Medicine and Allied Sciences, DRDO, Ministry of Defence, Brig SK Mazumdar Marg, Delhi, 110054, India.

² Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, NH- 58, Baghpat Crossing, Partapur Bypass Road, Meerut, 250005, India.

³ To whom correspondence should be addressed. (e-mail: sharma.nitin0909@gmail.com)

for colon drug delivery due to their pH-sensitive characteristics which permit release of only a limited amount of drug into the upper GIT and the rest into the colonic region. The pH-dependent drug release behavior of these polysaccharides has been attributed to the presence of hydrophilic groups on the polymeric backbone which cause electrostatic repulsion at the specific pH (9). Additionally, degradation of polysaccharide by colonic microflora permits complete drug release at a desired site and makes it a suitable choice as excipient for colonic drug delivery (10).

The present work describes the tablet formulation from a novel plant polysaccharide derived from *Trigonella foenum-graecum* (FG) that belongs to the family *Fabaceae*. Chemically speaking, polysaccharide consists of a high portion of galactomannan (73.6%). The polysaccharidal main chain is composed of β -linked D-mannopyranose, which is substituted at the 6th position with a single residue of α -linked D-galactopyranose. The polysaccharide has a molecular weight around 30,000 Da (11). This polysaccharide has already been investigated as an excipient in various pharmaceutical formulations such as gelling agents, tablet binders, disintegrators, suspending agents, and rate-controlling polymers (12). To the best of our knowledge, application of polysaccharide in colon-specific drug delivery has not been investigated so far. Therefore, in the present study, FG polysaccharide has been investigated for its potential role in the delivery of MNZ to the colon.

MATERIALS AND METHODS

Materials

MNZ was supplied as a gift sample from Plethico Pharmaceutical Pvt. Ltd., Indore, India. Fresh seeds of FG were authenticated by Dr. N.K. Dubey (Professor) of the Department of Botany, Banaras Hindu University, Varanasi, India (Voucher specimen No. Malv/2013/07). Technetium-99m (^{99m}Tc) for radiolabeling was procured from the Bhabha Atomic Research Centre, Mumbai, India, and stannous chloride (SnCl_2) was purchased from Sigma Aldrich (St. Louis, MO). Other ingredients used were of analytical grade and purchased from Central Drug Laboratory, New Delhi, India, and Sigma Aldrich (St. Louis, MO).

Polysaccharide Extraction and Purification

Dry polysaccharide was extracted as per procedure mentioned in our previous publication with slight modification (13). Briefly, 100g FG seeds were soaked in demineralized water (1 L) and then boiled with continuous stirring. The obtained mucilaginous slurry was kept overnight to allow settling of undissolved material. Supernatant was deproteinized using a pronase digestion method and a trichloroacetic acid (TCA) method. Centrifugation at 5000 rpm for 15 min separated the water soluble polysaccharide and the supernatant was dialyzed (cutoff M_w 3500 Da) against distilled water for 3 days. Product was concentrated to 1/3rd its volume on a rotary evaporator (RE301, Yamato, Japan). Precipitation of polysaccharide was done in acetone at a ratio of 1:3 mucilage to acetone after cooling. White precipitate was obtained after repeated washing, which was

dried in a hot air oven at 50–60°C until completely dry. The dried material was powdered and kept in a desiccator until further use. The obtained percentage yield of FG mucilage was 8.2% w/w.

Fourier Transform Infrared Spectroscopy

Equimolar quantity of MNZ and extracted polysaccharide (FG) were mixed together. The mixture was converted into powder using an agate mortar. Finally, the fine powder was stored in an air tight container for 30 days. KBr pellet was made with this sample using a hydraulic press. The KBr pellet containing the drug was scanned using a Fourier transform infrared (FTIR) spectrophotometer to confirm its presence in the pellet (Shimadzu, Kyoto, Japan).

Swelling Study

In order to determine pH sensitivity of extracted plant polysaccharide, swelling study was performed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Both media were prepared as per the method given in 34th United States Pharmacopeia (USP 2011). The dried sample of FG polysaccharide (1 g) was taken in a 25mL glass measuring cylinder. Two such cylinders were prepared and both types of simulated fluids were then added to them individually. This mixture was shaken for 10 min and left undisturbed for 3 h at 25°C. The experiment was repeated thrice and the average weight of swollen polysaccharide was placed in Eq. (1) to calculate the swelling ratio (SR) of polysaccharide in both the simulated fluids.

$$\text{SR} = (W_t - W_0) / W_0 \times 100 \quad (1)$$

where W_t is the weight of the swollen mass and W_0 is the weight of the dried sample.

Cell Viability Study of FG Polysaccharide

In order to determine cell viability of the extracted polysaccharide, MTT assay was performed. HT-29 cell lines were maintained in the media consisting of 10% fetal bovine serum (FBS) and 100 U/mL penicillin to prevent bacterial growth, and humidified under a CO_2 atmosphere at constant temperature ($37 \pm 0.5^\circ\text{C}$). Incubated cells were exposed to different dilutions of polysaccharide sample (10, 20, and 30 μg respectively) into 24-well plates containing 100 μL of the respective media at a plating density of 1×10^5 cells/well. Cells were carefully washed using saline to remove sample solution. Finally, a 5 mg/mL solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) was added (200 μL /well). The same method was followed to prepare a control sample of cells without treating them with the polysaccharide solution. Absorbances of test and control samples were recorded at 570 nm. Finally, percentage cell viability was calculated using Eq. (2).

%cell viability

$$= \frac{\text{absorbance of test sample}}{\text{absorbance of control}} \times 100 \quad (2)$$

Preparation of Tablets

Core Tablets

Core tablet of MNZ was prepared by a direct compression method. Composition of each core tablet was MNZ (100 mg), MCC (88 mg), croscarmellose (20 mg), magnesium stearate (1 mg), and talc (1 mg). All ingredients were mixed thoroughly and passed through a mesh sieve (Number 80). The mixed dry powder was compressed on a 8-mm flat round punch on an 8 station tableting machine (Cadmach, India) by applying 10–20 KN force.

Compression-Coated Tablet

Core tablets of MNZ (with a total weight of 210 mg) were prepared by the direct compression method using 400 mg of powder mixture of FG polysaccharide and microcrystalline cellulose (Table I). For compression coating, 200 mg of coating powder was filled in the die cavity and the core tablet was placed in the center and then the remaining quantity of coating powder was transferred into the cavity. Coating compression was performed around the core tablet using 12 mm of flat punch on the 8 station tableting machine by applying 10–20 KN compression force.

Tablet Characterization

Various tablet parameters such as thickness, percent weight variation, hardness, and drug uniformity test were evaluated for all the prepared batches. Tablet thickness was determined precisely with the help of a vernier caliper (Mitutoyo, Japan). For weight variation, the average weight of 20 tablets was recorded and individual weight of tablets was compared to the average weight. Hardness of tablets was determined using a Monsanto tester and represented as kilograms per cubic centimeter. Friability of prepared tablets was evaluated using a friability test apparatus (Electro Lab, Mumbai India) by following standard protocol.

In order to determine drug uniformity test, ten tablets from each batch were randomly taken and crushed. The crushed powder equivalent to 100 mg MNZ was transferred to a volumetric flask having 0.1 M hydrochloric acid (pH 1.2). The content was shaken occasionally for dispersion of MNZ into the media. After filtration, the samples were analyzed for MNZ concentration using a UV spectrophotometer (Shimadzu 1700, Japan) against blank.

In Vitro Drug Release Studies

Dissolution studies were performed using a USP XIII paddle-type dissolution test apparatus (Model-TDT-08L, Electrolab Mumbai, India) at a rotation rate of 100 rpm temperature of $37 \pm 0.5^\circ\text{C}$. The prepared batches of MNZ tablets were analyzed for their drug release behavior in various physiological conditions of GIT to simulate mouth to colon transit as reported by Klein *et al.* (14) with moderate changes in parameters. For the initial 2 h, 0.1 N hydrochloric acid dissolution media (900 mL, pH 1.2) was used. Thereafter, the pH of the dissolution media was adjusted to pH 6.8 by potassium dihydrogen phosphate and sodium hydroxide and the procedure continued for the next 4 h. Finally, the dissolution study was performed at pH 7.4 by adjusting the pH using the method discussed above and supplemented with 4% (*w/v*) of rat cecal material. Rat cecal material was added into the dissolution media in order to mimic colonic conditions. Fresh cecal material was collected approximately 30 min prior to the commencement of dissolution study, as per method reported by Newton *et al.* (15). Briefly, eight albino rats were sacrificed by a cervical dislocation method and dissected and their abdomen was opened. Immediately, the cecal part was removed from intestine material and suspended in phosphate-buffered saline (pH 6.8) to give a final cecal concentration dilution of 4% (*w/v*) bubbled with carbon dioxide. Dissolution media (2 mL) were withdrawn at 1 h interval and equal quantity of phosphate-buffered saline (pH 6.8, pH 7.4) was added into the media to maintain the same volume of the dissolution media. The sample was processed by filtration and drug concentration was determined using a UV-visible spectrophotometer.

Drug Release Mechanism

To estimate release mechanism of MNZ, drug release data were fitted into the following release models.

Table I. Composition of Coat Material of *Trigonella foenum-graecum* Polysaccharide-Based Colon Tablets

Formulation code	FG* polysaccharide (mg)	Microcrystalline cellulose (mg)	Magnesium stearate (mg)	Talc (mg)
T1	400	–	2.5	2.5
T2	390	10	2.5	2.5
T3	380	20	2.5	2.5
T4	370	30	2.5	2.5
T5	360	40	2.5	2.5
T6	350	50	2.5	2.5

**Trigonella foenum-graecum* (Fenugreek, FG)

Zero-Order Kinetics

According to this model,

$$Q_t = Q_0 + K_0 t$$

where Q_t is the quantity of the drug dissolved in time t , Q_0 is the initial quantity of drug, and K_0 is the zero-order release constant.

First-Order Kinetics

According to this model,

$$\log Q_t = \log Q_0 + K_1 t / 2.303$$

where Q_t is the quantity of drug released in time t , Q_0 is the initial quantity of drug, and K_1 is the first-order release constant.

Higuchi Kinetics

According to this model,

$$M_t / M_\infty = k_H t^{1/2}$$

where M_t and M_∞ are amounts of drug release at time t and infinite, and k_H is the Higuchi constant.

Kossmeyer-Peppas

This model is described by the equation

$$[M_t / M_0] = K t^n$$

where M_t is the fraction of drug release at time t , M_0 is the fraction of drug release at initial time, K is Kossmeyer-Peppas constant, and “ n ” is the release exponent.

Gamma Scintigraphy

Radiolabeling of MNZ

MNZ was radiolabeled with ^{99m}Tc -pertechnetate as per protocol followed by Sharma *et al.* (5). Briefly, drug was shifted into a test tube containing a mixture of 1 mL $\text{SnCl}_2 \cdot \text{H}_2\text{O}$ solution (15 mg/mL) in 1 M HCl, 2.5 mL NaCl (0.9% w/v), 2.5 mL CH_3COONa (100 mg/mL), and NaTcO_4 (of about 20 MBq activity). The drug suspension was vortexed and centrifuged for 20 min at 3000 rpm. Then, the supernatant was separated and the radiolabeled drug was stored after freeze drying. Labeling efficiency of MNZ with the radioactive material was determined using paper chromatography. Acetone was used as the mobile phase and instant thin layer chromatography strips as stationary phase. Radioactivity was measured in a gamma counter (CAPRAC-R, Capintec, USA). Core tablet of radiolabeled MNZ and

further compression coating of extracted polysaccharide was done as per the method discussed above.

Targeting Efficiency

Colon targeting efficiency of the prepared tablets was determined in human volunteers using gamma scintigraphy. Six healthy adult volunteers, aged between 20 and 30 years, were selected for the study. The protocol was approved by the institutional human ethical committee (vide no: IEC/INM/15-16/1-13) and prior written consent of human volunteers was taken. During the study period, all volunteers were restricted to take any other medication or therapy. A written advisory was also given to all volunteers before the beginning of the study.

Radiolabeled MNZ tablet (containing 300 μCi of technetium pertechnetate complexes to MNZ) was orally administered in human volunteers with filtered portable water after 1 h of standard meal. The volunteers were asked to be moderately active during the study period. Static images (60 s/image) were captured under gamma camera (Siemens gamma camera) at 1, 2, 3, 4, 6, 8, 12, and 24 h time points.

RESULTS

FTIR Study

FTIR spectra of pure drug (MNZ), FG polysaccharide, and physical mixture are presented in Fig. 1. Spectra of MNZ (Fig. 1(a)) showed O–H stretching at 3239.47 cm^{-1} and C–H stretching at 3102.38 cm^{-1} . Peak at 3017.29 and 1542.39 cm^{-1} was due to asymmetric stretching of $-\text{CH}_2$ and asymmetric stretching due to the NO_2 group. Similarly, the spectra of FG polysaccharide (Fig. 1(b)) showed a broad absorption peak at 3346.37 and 2930.48 cm^{-1} due to O–H and C–H stretching respectively. Peaks appearing at 1410.38 and 1021.73 cm^{-1} could be assigned to symmetric $-\text{CH}_2$ bending vibrations and $-\text{C}-\text{O}-\text{C}-$ stretching respectively.

Swelling Study

Swelling study was performed to determine pH-dependent swelling behavior of FG polysaccharide. Result showed that FG polysaccharide possesses a pH-dependent swelling behavior. It was observed that swelling ratio of polysaccharide was 162 and 384% in SGF and SIF respectively.

Cell Viability Study

Absorbance data of test and control sample were introduced into Eq. (2). Results of percent cell viability of FG for 10, 20, and 30 μg concentrations was evaluated and found to be 85.97, 91.31, and 87.82% respectively, as compared to the control.

Tablet Characterization

Both core and compression-coated tablets were examined for various parameters. Core tablets were formulated in such a way so as to disperse immediately (within 1 min) in the

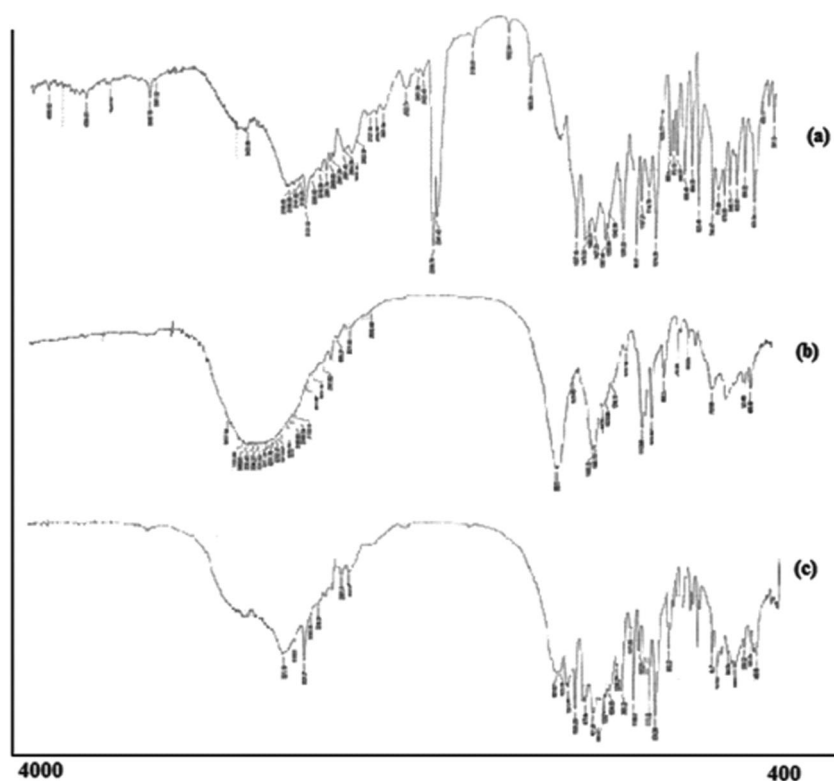


Fig. 1. FT-IR spectra of **a** metronidazole, **b** polysaccharide of *Trigonella foenum-graecum*, and **c** physical mixture

dissolution media. Obtained data of compression coated tablets is given in Table II. The average weight of the prepared tablets was found between 604.66 ± 8.39 and 616.90 ± 4.34 mg. The value of percent weight variation of all batches was found within the acceptable limits of United States Pharmacopeia (16). The thickness and diameter of the tablets were in the range of 4.86 ± 0.28 to 4.91 ± 0.29 mm and 12.06 ± 0.02 to 12.10 ± 0.02 mm respectively. Similarly, hardness of tablets prepared for all the batches was found to be in the range of 0.0651 ± 0.0031 to 0.0745 ± 0.0036 KN. Friability was less than 1% for all the batches which revealed the acceptable limit of the parameters. For all the formulations, the percentage drug content was found in the satisfactory range of 101.16 to 104.37% w/w.

In Vitro Dissolution Studies

The release patterns of MNZ from different batches of tablets in simulating gastrointestinal tract conditions are shown in Fig. 2. It was observed that all the prepared tablets released less than $3.37 \pm 0.69\%$ MNZ in the first 2 h of study at pH 1.2 whereas release rate increased significantly up to $25.39 \pm 3.69\%$ at pH 6.8. It was also observed that in the presence of cecal material, release of MNZ was significantly increased ($p < 0.05$) after the 6-h period.

Figure 2 shows the effects of the ratio of FG polysaccharide and microcrystalline cellulose on the release of MNZ from the tablet. It was found that the concentration of FG polysaccharide in the tablet formulation may affect the drug release rate significantly ($p < 0.05$). Drug release was found to increase by replacing the polysaccharide from microcrystalline cellulose. Formulation T6 released $97.21 \pm 5.38\%$ w/w of

the drug up to 22 h of dissolution study whereas formulations T1, T2, T3, T4, and T5 released 62.24 ± 6.37 , 71.85 ± 11.61 , 75.38 ± 11.02 , 79.37 ± 7.29 , and $87.93 \pm 5.41\%$ respectively. As per the data of dissolution study, formulation T6 was selected for further *in vivo* scintigraphy study.

Drug Release Kinetics

Release mechanism of MNZ from the prepared tablets was determined by modeling the obtained release data as per various release kinetic models available. The obtained results of drug release kinetics study are summarized in Table III.

Gamma Scintigraphy Study in Human Volunteers

In order to determine the colon-specific targeting efficiency of the prepared tablets, gamma scintigraphy was performed. Anterior images of the human abdomen were taken in supine position to assess the mobility of radiolabeled MNZ tablets. Scintigraphic images (Fig. 3) at different intervals indicate that the gastric and small intestinal transit time of radiolabeled tablet was 2–3 ($2.2 \pm .2$) h and 5–8 ($7.2 \pm .4$) h respectively. Moreover, in all volunteers, the tablets took approximately 6–8 h to arrive in the colonic region. As predicted, the tablets maintained their structural integrity with negligible or slight sign of radiolabeled MNZ release in the upper GIT. Tablets began to release the tracer at the ileocecal junction, indicating the degradation of the outer coat of FG polysaccharide. Radiolabeled drug content release started after 8 h of oral administration and drug distribution was observed in the entire colon up to 24 h in all volunteers. Images obtained from *in vivo* gamma scintigraphy indicated

Table II. Post Compression Parameters of *Trigonella foenum-graecum* Polysaccharide-Based Colon Tablets

Formulation code	Average weight (mg)	Weight variation (% w/w)	Thickness (mm)	Diameter (mm)	Hardness (KN)	Friability (%)	Drug content (% w/w)
T1	607.48 ± 8.12	5.49 ± 0.36	4.88 ± 0.32	12.06 ± 0.02	0.0656 ± 0.0024	0.38 ± 0.05	103.54 ± 4.61
T2	605.79 ± 12.36	7.03 ± 0.58	4.87 ± 0.21	12.10 ± 0.02	0.0745 ± 0.0036	0.58 ± 0.04	101.16 ± 2.60
T3	611.71 ± 12.72	8.22 ± 0.37	4.89 ± 0.26	12.07 ± 0.01	0.0695 ± 0.0017	0.16 ± 0.05	103.22 ± 3.73
T4	604.66 ± 8.39	4.38 ± 0.31	4.86 ± 0.28	12.09 ± 0.02	0.0702 ± 0.0028	0.32 ± 0.02	104.37 ± 1.04
T5	616.90 ± 4.34	9.49 ± 0.62	4.91 ± 0.29	12.07 ± 0.01	0.0651 ± 0.0031	0.41 ± 0.04	102.44 ± 8.38
T6	614.83 ± 6.67	7.89 ± 0.46	4.87 ± 0.30	12.08 ± 0.01	0.0733 ± 0.0025	0.17 ± 0.03	102.83 ± 5.48

that FG polysaccharide-based formulation successfully demonstrated colon-targeted drug delivery. The developed approach could be a safe, nontoxic, and effective method for drug delivery specifically to the colonic region for the treatment of disorders like amoebiasis.

DISCUSSION

FTIR spectra of the drug, FG polysaccharide, and mixture of drug and extracted polysaccharide were recorded. These indicated the presence of all the characteristic peaks of MNZ in the spectrum of the physical mixture (Fig. 1(c)), with no significant interaction between FG polysaccharide and MNZ. The study indicated that the MNZ remain physically and chemically stable with FG polysaccharide.

Swelling study revealed that swelling ratio of FG polysaccharide was significantly high ($p < 0.05$) in SIF as compared to the SGF, therefore indicating the latter as a suitable choice as excipient of tablets for colon drug delivery. Higher swelling of FG polysaccharide in SIF could be attributed to the complete ionization of several hydrophilic groups attached in the polysaccharidal chain, which provide electrostatic repulsion and further allow entering of large quantity of water deeply inside the polymeric structure (17,18). In case of SGF, acidic environment of media was unable to ionize the polymeric hydrophilic groups which led to a comparatively slower swelling rate.

It is desirable to access the safety profile of any novel material before intended biological use. With the same objectives, cytotoxicity of FG polysaccharide was determined using MTT assay. The present study indicated that none of the dilutions of extracted polysaccharide possess cytotoxic effects on HT-29 cell line and could therefore be considered as safe excipients for use in any drug delivery system. Different batches of MNZ tablets were prepared using FG polysaccharide as coating material. All post compression parameters were within prescribed limits which concludes that the present method can be considered feasible for the preparation of FG polysaccharide-based colon tablets.

To verify the colon targeting characteristics of all variants of FG polysaccharide-based tablets, dissolution study was performed in media with different pH values, with and without rat cecal material, to simulate the actual physiology of the colon. Dissolution data without rat cecal material is not included in the manuscript. Figure 2 indicates that pH of dissolution media has a great effect on the release behavior of drug. Drug release data were in accordance with the swelling property of extracted polysaccharide where it swells less in SGF compared to SIF. According to Junginger *et al.*, polymer of high swelling capacity allowed media to enter deeply which causes relaxation of the complex impermeable polymeric network (19). Now after swelling, this semi permeable network allowed the passage of drug from the formulation to the dissolution media. You *et al.* (20) demonstrated an increase of drug release at intestinal pH due to the cleavage

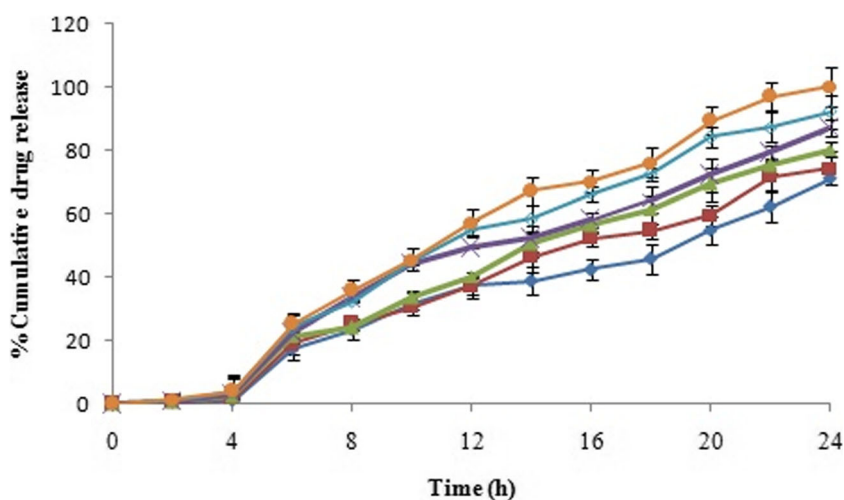


Fig. 2. Release profile of metronidazole from different batches of *Trigonella foenum-graecum*-based colon tablets [T1 (◆), T2 (■), T3 (▲), T4 (×), T5 (◇), T6 (●)]

Table III. Values of Kinetic Rate Constant (k), Correlation Coefficient (R^2), and Release Exponent (n) of Metronidazole from *Trigonella foenum-graecum* Polysaccharide-Based Colon Tablets

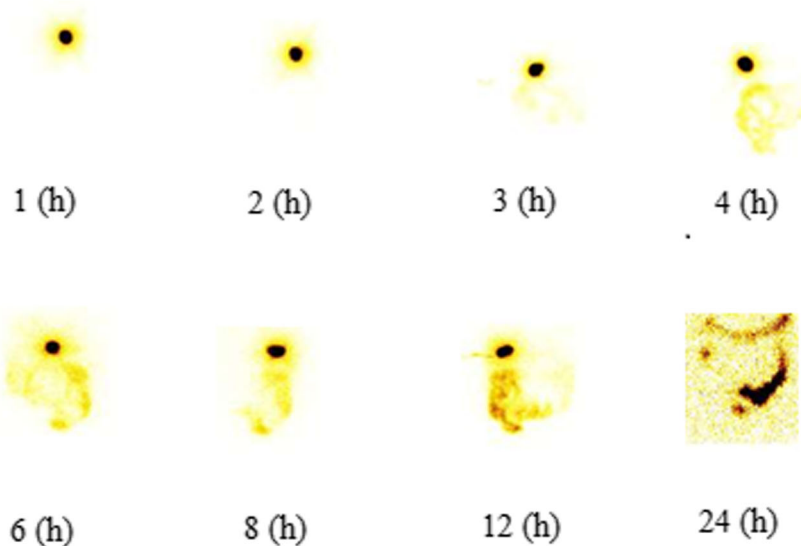
Formulations	Zero-order		First-order		Higuchi		Korsmeyer-Peppas		
	k	r^2	k	r^2	K	r^2	k	r^2	n
T1	3.273	0.9883	-0.052	0.9832	8.739	0.9836	3.843	0.9725	1.299
T2	3.745	0.9824	-0.063	0.9892	9.681	0.9281	3.515	0.985	1.236
T3	3.028	0.9735	-0.052	0.9833	9.729	0.9395	3.483	0.982	1.454
T4	4.379	0.8827	-0.102	0.9761	10.244	0.9478	3.972	0.961	1.394
T5	4.3946	0.8927	-0.131	0.9783	10.599	0.9382	3.853	0.973	1.275
T6	4.9355	0.8882	-0.313	0.8531	10.046	0.9853	3.410	0.963	1.163

of glycosidic bond from polysaccharide material which causes migration of drug into the dissolution media. The release study clearly indicated that the tablet maintained its physical structural integrity at both pH range of media (1.2 and 7.4). However, in the presence of rat cecal material, the outer surface of tablets started to degrade and release of MNZ increased. The reason for increased release of MNZ in the presence of cecal material may be due to the breakage of biodegradable material under the influence of activity of enzymes produced by polysaccharide-degrading bacteria present in the cecal material. Comparatively, batch T6 released the drug in the desired range of time period and was therefore considered as a choice for further study. The present results indicated that the FG polysaccharide had promising applications in colon drug delivery systems.

In order to determine exact drug release mechanism from prepared formulation, release data were fitted to release kinetic models. The appropriate kinetic model was selected on the basis of goodness of fit. Values of kinetic models indicated that the release of MNZ followed first-order release as the cumulative percent release vs. time plots was linear, which indicated the drug release from prepared tablets was independent of the concentration of FG polysaccharide. Further, release data was fitted into Higuchi's equation. All plots were found linear with

correlation coefficient values (r^2) in the range of 0.9281 to 0.9853 (Table II), which indicated a diffusion-controlled drug release from the prepared formulations. Finally, to know the mechanism of diffusion (suggested by Higuchi's model), the release data was applied into Korsmeyer-Peppas' model. The n value of all formulations was found to be more than 0.89 which indicated a super case-II transport (21).

Gamma scintigraphy is a validated modality that predicts the *in vivo* performance of dosage form through a non-invasive mode of operation. By incorporating radioactive material into the delivery system, it is possible to visualize GI transit pattern of a developed delivery system. This technique enables the assessment of critical performance parameters that the *in vitro* technique attempts (22). In the present study, gamma scintigraphy images clearly indicated that prepared tablet of FG polysaccharide follows a normal transit pattern from the upper GIT to the colon. Gamma scintigraphy images evidenced that the prepared tablets reached the colonic area within 6–8 h and allowed majority of drug release at the colon up to a 24-h time period. Gamma images were also in accordance with the minimum drug release at outer parts of GIT. Thus, it can be presumed that the core tablet disintegrates immediately and releases the radiolabeled MNZ once pH-dependent swollen FG polysaccharide coat gets degraded by the bacteria present in the colonic region. The combined response of

**Fig. 3.** Gamma scintigraphy images of *Trigonella foenum-graecum* polysaccharide-based colon-specific tablet in a human volunteer up to 24 h

FG polysaccharide (pH-dependent swelling property and polysaccharide degradation in the presence of colonic bacteria) helps to achieve colon-targeted drug delivery.

CONCLUSION

The study was successful in order to develop a compression coating tablet using *Trigonella foenum-graecum* polysaccharide. MTT assay indicated its safety profile with the biological tissues. FG polysaccharide-based colon tablets possess tableting behavior within the prescribed range. Dissolution data and gamma scintigraphy images indicate that FG polysaccharide prevents the rapid drug release in the upper part of GIT and at the same time ensures complete release of the entrapped drug in the colon. The present study successfully demonstrates that FG polysaccharide may be considered an effective and safe excipient in order to provide colon-specific drug delivery. However, the applications of the study are not limited to the present outcomes but can also be used in other physiological conditions which require colon-specific drug delivery. Safety, biocompatibility, and low extraction cost make the FG polysaccharide with great market foregrounds an alternate of synthetic polymers.

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COMPLIANCE WITH ETHICAL STANDARDS

The protocol was approved by the institutional human ethical committee (vide no: IEC/INM/15-16/1-13) and prior written consent of human volunteers was taken.

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