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EFFECT OF PROBIOTICS ADDITION AND SHELLAC COATING ON *IN-VITRO* RELEASE OF FLUOROURACIL FROM GUAR GUM MATRIX TABLETS FOR COLON SPECIFIC DELIVERY

Ghosh PK[1], Thakur Namarta[2], Rathore MS[3]*

[1]KEEGAD Biogen Pvt. Ltd., A-8, Third Floor, Narina Industrial Area Phase II, New Delhi, India

[2]CT Institute of Pharmaceutical Sciences, Jalandhar, Punjab, India

[3*]MM College of Pharmacy, MM University, Mullana, Ambala, Haryana, India.

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***Correspondence for Author**
Dr Mahendra Singh Rathore
MM College of Pharmacy, MM University, Mullana, Ambala, Haryana, India

ABSTRACT

Aim of present research work was to evaluate the effect of 5-fluorouracil (5-FU) release from guar gum matrix tablet upon addition of probiotics and shellac coating for development of efficient colon specific drug delivery system. Guar gum matrix tablets (40% guar gum) were prepared and evaluated for drug release. The matrix tablets were formulated with three different probiotics (*Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Lactobacillus sporogenes*) and drug release was studied. Further, the tablets were also coated with varying concentration of shellac solution (5% or 10% or 15 % w/v). The results showed that tablets coated with 10 % shellac solution were able to retard the drug release for first five hours while these released major portion (above 80%) in phosphate buffer saline (pH 6.8) at the end of 24 hours in the *in-vitro* drug release studies. From above work it may be concluded that matrix tablets coated with 10 % shellac solution that contain probiotics may be employed successfully for colon specific delivery to ensure maximum drug release at colon even in patients with disturbed gastrointestinal microflora.

Key Words: Guar Gum, Probiotics, Colon, Drug Delivery.

INTRODUCTION

Colon is considered as a potential site for delivering therapeutic agents in clinical conditions such as colon cancer, inflammatory bowel disease, ulcerative colitis and Crohn's disease. [1-2]

Several attempts have been made in order to deliver drugs to colon for treating its disorders by oral route in tablet form. Digestions of biodegradable polymers resulting in drug release and coating the tablets with polymers are fewer successful approaches among them.[3] Fluorouracil (5-FU), is a pyrimidine analog which is a thymidylate synthase inhibitor; inhibition of this enzyme results in blocking the synthesis of thymidine, a nucleoside required for the replication of DNA. 5-FU is an antimetabolite which is used in different types of cancers and stood alone as the only chemotherapeutic agent with clinical activity against colorectal cancer for several decades.[4] Intravenous administration of 5-FU is associated with several cytotoxic effects.[5] Henceforth, attempts have been made for its colon specific delivery through oral route.[6-7] Site specific delivery to colon would be an advantage in order to reduce the side effects of this potential drug candidate.

Drug delivery to colon through oral route faces several challenges. Guar gum has been extensively studied for colon drug delivery. [8-11] Guar gum based tablets of 5-FU in healthy human volunteers showed delayed absorption, decreased absorption rate and decreased peak concentration in plasma compared to immediate-release tablets [12]. The problems associated with colonic drug delivery through oral route include premature drug release from the medicament, possibility of failure of digestion of polysaccharides in colon in certain conditions etc [13-14]. To overcome the problem of polysaccharide digestion failure, use of probiotic assisted colon specific delivery of diclofenac from guar gum matrix tablets has already been reported through in-vitro studies.[15] Combining two or more approaches could be useful in order to meet the drug delivery challenges. Coating of the matrix tablets with material like shellac would be further useful in assisting the drug delivery to colon.[16] Keeping the above in view, attempts have been made in the present research work to develop shellac coated guar gum matrix tablet containing probiotics to get optimal delivery of 5-FU to colon through oral route.

MATERIALS AND METHODS

Fluorouracil (5-FU), *Lactobacillus acidophyllus* (LA), *Lactobacillus sporogenes* (LS), *Bifidobacterium bifidum* (BB) and Shellac were procured from KEE GAD Biogen Pvt. Ltd., New Delhi, India. Guar gum was obtained from SD Fine Chemicals,Mumbai. Other materials used in the study such as lactose, talc, magnesium stearate were of analytical grade. Double beam UV-visible Spectrophotometer (UV-1800, Shimadzu Corporation, Japan), Brookfield viscometer (RVT/282889, Brookfield, USA) were used for analysis of samples.

***In-Vitro* digestion studies of guar gum by bacterial spores**

Accurately weighed 2gm of guar gum powder was transferred slowly in the beaker containing 200ml of warm distilled water. The slurry was mixed on magnetic stirrer with magnetic bead for 30 minutes and kept at 37^0C ± 2^0C in incubator for 24 hours. After 24 hours, in each 200ml guar gum (1 percent w/v) slurry, different bacterial spores (*Bifidobacterium bifidum, Lactobacillus acidophilus* and *Lactobacillus sporogenes*) were added (1 percent w/v) in different beakers and incubated at 37^0C ± 2^0C in incubator. At different time interval the change in pH and viscosity were measured using calibrated pH meter (Remi) and Brookfield viscometer.

Formulation of matrix tablets of 5-FU with guar gum

Matrix tablets of 5-FU (50 mg) using analytical grade guar gum were prepared by wet granulation method. Lactose was used as diluent and the mixture of talc and magnesium stearate at 2:1 ratio was used as lubricant. Guar gum was sieved (sieve no. 60) separately and mixed with 5-FU (sieve no. 100) and lactose (sieve no. 60). The composition of different formulations is shown in Table 1. The powders were blended and granulated with water. The wet mass obtained was then passed through sieve no. 14 and wet granules were dried at 50°C for 2 h. The dried granules were passed through a sieve no. 16 and were lubricated with a mixture of talc and magnesium stearate (2:1). The lubricated granules were compressed at compression force 5000-6000 kg using 12-mm-round concave-convex punch on eight-station rotary tablet machine (M/s Kambert Machinery Co. Pvt. Ltd., Ahmedabad, India). Uncoated tablets of 5FU were coated with 5% or 10% or 15% of shellac solution in ethanol and isopropylalcohol (1:1) by dip coating method.

***In vitro* dissolution studies**

In vitro dissolution studies for all coated and uncoated matrix tablet formulations were performed by using USP dissolution test apparatus (Apparatus 1, Basket type, 37°C) at 100 rpm for 2 hr in 0.1 N HCl (900 ml).Then the dissolution medium was replaced with pH 6.8 phosphate buffer (900 ml) and tested for next 22 hr. At different time intervals a 10 ml of the sample was taken and analyzed for 5-FU content at 266 nm by double beam UV-visible spectrophotometer. A 10 ml fresh dissolution medium was added to make the volume after each sample withdrawal.

DISCUSSION

Table 1: Formulation of guar gum tablets (uncoated)

S.No.	Ingredients	Amount (% of Total Tablet Weight)
1	Fluorouracil (5-FU)	10
2	Guar gum	20 or 40 or 50 percent
3	Probiotics *(L. sporogenes / L. acid ophyllus / B. bifidum)*	1:1 ratio to guar gum
4	Lactose	Diluent to make total weight of tablet
5	Magnesium stearate	1%
6	Talc	2%

Total tablet weight kept was 500 mg. Weight of tablets having 50% guar gum was kept at 564 mg to accommodate requisite amount of ingredients**.**

Table 2: Comparison in viscosity change in guar gum (1% dispersion in water) by different probiotics (values are Mean±SEM, n=3), viscosity in centipoises

Time (hours)	*Bifidobacterium bifidum*	*Lactobacillus acidophillus*	*Lactobacillus sporogenes*	Without probiotics (Control)
0	2760.00 ± 0.00	2760.00 ± 0.00	2760.00 ± 0.00	2760.00 ± 0.00
2	2750.00 ± 5.78	2746.00 ± 3.33	2750.00 ± 5.78	2760.00 ± 0.00
4	2736.67 ± 3.33	2733.33 ± 3.33	2730.00 ± 5.78	2760.00 ± 0.00
6	2723.33 ± 3.33	2720.00 ± 5.78	2710.00 ± 5.78	2760.00 ± 0.00
7	2630.00 ± 11.56	2610.00 ± 5.78	2686.67 ± 8.82	2760.00 ± 0.00
8	1906.67 ± 12.03	2516.67 ± 12.03	2546.67 ± 12.03	2753.33 ± 3.33
9	1800.00 ± 5.78	2543.33 ± 24.06	2436.67 ± 17.65	2746.67 ± 3.33
10	1730.00 ± 17.34	2276.67 ± 8.82	1860.00 ± 17.34	2726.67 ± 3.33
12	1600.00 ± 17.34	2080.67 ± 8.82	1726.67 ± 8.82	2713.33 ± 3.33
14	1430.00 ± 11.58	1950.00 ± 11.56	1590.00 ± 5.78	2703.33 ± 3.33
16	1300.00 ± 5.78	1800.00 ± 5.78	1426.67 ± 14.54	2696.67 ± 3.33
18	1080.00 ± 5.78	1690.00 ± 5.78	1313.33 ± 12.03	2683.33 ± 3.33
20	980.0± 5.78	1566.67 ± 34.84	1233.33 ± 38.48	2673.33 ± 3.33
22	860.00 ± 15.29	1466.67 ± 17.65	1106.67 ± 29.66	2670.00 ± 5.78
24	783.33 ± 17.66	1376.67 ± 38.48	1040.00 ± 32.18	2656.67 ± 6.67

Table 3: Cumulative % Release of 5FU from guar gum matrix tablet (20%,40%, 50%) without and with Probiotics (BB,LA and LS) in 1:1 ratio with guar gum (values are Mean±SEM, n=3)

Sr.No.	Time hours	Guar Gum Tablets Without Probiotic	Guar gum Tablet with BB	Guar gum Tablet with LA	Guar gum Tablet with LS
20% Guar Gum	2	33.5400 ±0.62761	64.35±0.6345	45.40±0.5690	40.09±0.9847
	5	61.0067±0.8221	82.94±0.1203	72.94±0.0287	67.76±0.4783
40 % Guar Gum	2	33.06±0.4536	48.03±0.3245	37.19±0.7483	35.66±0.8473
	5	59.28±0.3876	62.53±0.9873	57.14±0.8365	55.87±0.6474
50% Guar gum	2	29.86± 0.5673	37.10±0.5467	30.28±0.2536	31.19±0.9102
	5	48.35± 0.9765	57.00±0.2313	51.37±0.2987	52.86±0.8474

Table 4: Cumulative % release of 5-FU from matrix tablets containing 40 percent guar gum coated with 5 percent shellac solution with or without probiotics (values are Mean±SEM, n=3)

Sr.No.	Time hours	Guar Gum Tablets Without Probiotic	Guar gum Tablet with BB	Guar gum Tablet with LA	Guar gum Tablet with LS
20% Guar Gum	2	33.5400 ±0.62761	64.35±0.6345	45.40±0.5690	40.09±0.9847
	5	61.0067±0.8221	82.94±0.1203	72.94±0.0287	67.76±0.4783
40 % Guar Gum	2	33.06±0.4536	48.03±0.3245	37.19±0.7483	35.66±0.8473
	5	59.28±0.3876	62.53±0.9873	57.14±0.8365	55.87±0.6474
50% Guar gum	2	29.86± 0.5673	37.10±0.5467	30.28±0.2536	31.19±0.9102
	5	48.35± 0.9765	57.00±0.2313	51.37±0.2987	52.86±0.8474

Table 5: Cumulative % release of 5-FU from matrix tablets containing 40 percent guar gum coated with 10 percent shellac solution with or without probiotics . (values are Mean±SEM, n=3)

S.No.	Time (hours)	Guar Gum Tablets Without Probiotic	Guar gum Tablet with BB	Guar gum Tablet with LA	Guar gum Tablet with LS
1.	2	1.4067±0.4582	2.6300±0.1721	1.3933±0.1727	3.3800±0.17211
2.	5	10.4333±0.6789	20.9067±0.7744	20.4600±0.6788	22.3067±0.53025
3.	7	19.9600±0.6053	40.9567±0.4670	32.3000±0.2268	43.5633±0.95662
4.	9	30.0800±0.7272	61.2733±0.2190	44.5700±0.6304	59.4533±0.28598
5.	12	48.3767±0.7473	88.6900±0.7455	62.3833±1.1573	81.8567±0.7264
6.	15	66.8747±0.5345	93.7433±0.5752	74.8533±0.5117	83.5167±0.2771
7.	18	78.5600±0.2286	94.4900±0.4981	79.2933±0.5060	91.8833±0.4863
8.	21	83.4333±1.1092	97.4500±0.7840	87.5533±0.4080	96.5233±0.7125
9.	24	83.5267±0.2773	99.3633±0.5396	95.5833±0.5364	99.4067±0.20463

Table 6: Cumulative % release of 5-FU from matrix tablets containing 40 percent guar gum coated with 15 percent shellac solution with or without probiotics . (values are Mean ± SEM, n=3)

Sr.No.	Time (hours)	Guar Gum Tablets Without Probiotic	Guar gum Tablet with BB	Guar gum Tablet with LA	Guar gum Tablet with LS
1.	2	0.98±0.8473	1.77±0.4784	1.27±0.8874	1.47±0.2234
2.	5	4.75±0.4839	5.96±0.7282	6.24±0.7383	5.70±0.1902
3.	7	9.66±0.5483	11.16±0.7783	10.21±0.1010	10.12±0.6575
4.	9	13.36±0.8373	15.57±0.9807	15.04±0.4673	14.92±0.3778
5.	12	18.16±0.6372	26.02±0.9033	25.14±0.1902	24.94±0.7585
6.	15	24.13±0.5273	29.26±0.7221	28.34±0.9876	28.36±0.8870
7.	18	28.01±0.5849	34.55±0.4788	33.42±0.9987	32.66±0.9684
8.	21	31.24±0.1023	43.23±0.4022	37.76±0.7332	34.12±0.4784
9.	24	35.15±0.4536	52.12±0.9478	48.17±0.2778	42.93±0.9393

In human intestine guar gum (a polysaccharide) is known to degrade by fermentation to short-chain fatty acids (SCFA) with evolution of Carbond dioxide (CO_2). The viscosity of the guar gum polysacchrides is likely to be reduced by deploymerization process with reduction in surrounding pH due to generation of SCFA and CO_2. These two parameters were taken as indicator to investigate the depolymerization of guar gum *in vitro* in presence of the spores of *Lactobacillus acidophyllus, Lactobacillus sporogenes, Bifidobacterium bifidum* and the results are shown in Table 2. The extent of breakdown of guar gum was influenced by the bacterial enzymes present as well as the chemical structure of guar gum; a fall in viscosity can be related to a reduction of polysaccharide chain length i.e. destruction of (1,4)-β-linked D-mannan backbone by microbial enzymes. [17] A fall in pH is caused by the production of short chain fatty acids as well as generation of CO_2, which indicates that fermentation has occurred. Thus the data suggested that a mixed population of colonic bacteria can produce extracellular enzymes that reduce the chain length of guar gum. Viscosity and pH reducing capacity was more in case of *Bifidobacterium bifidum* than all others spores used. The order of pH reduction by all probiotics was *Lactophilus acidophilus* > *Bifidobacterium bifidum* > *Lactobacillus sporogenes;* the order was selected on the basis of differences in the initial to the final pH. The order of viscosity reduction by all probiotics was *Bifidobacterium bifidum* > *Lactobacillus sporogenes* > *Lactophilus acidophilus*. The order was selected on the basis of differences in the initial to the final viscosity.

In vitro dissolution studies of 5FU from 20 or 40 or 50% guar gum tablets were performed and dissolution data is shown in table 3. In all cases the drug release rate was about 30% or more. However, the tablets formulated with probiotics showed comparatively higher % of drug release in first five hours. In colon specific formulation development the drug reslease is to kept minimized (preferably below 20%) in first five hours i.e. the residence time for formulation in upper gastrointestinal tract. Henceforth, coating of the formulation was desired to control the drug release. According to work done by Ghosh et.al. tablets of diclofenac with 40% guar gum concentration were selected for further studies in development of colon specific formulation.[14] Further studies (dissolution studies from coated tablets of shellac) in present research work were carried out with tablets of 40% guar gum concentration.

The 40% guar gum tablets with and without probiotics were coated with 5% or 10% or 15% shellac solution in 1:1 ethanol and isopropyl alcohol. Tablets of 5-FU coated with 5% shellac showed more than 20% release that was not desired (Table 4). Tablets coated with 10%

shellac showed the desired release characteristics as shown in table (Table 5). Shellac is a polymer that is not soluble in acidic environment, hence the 10% coat was capable to retard the 5-FU release in first 2 hour. In basic medium shellac dissolved and the same was reflected by increased release of 5-FU in subsequent hors when pH was about neutral i.e. phosphate buffer of pH 6.8. Spores usually take 7-9 hours to come in vegetative form and start digesting guar gum. A sudden increase in drug release after 7th hour is indicative of this fact (Table 4). The release rate was quite low in tablets coated with 15% shellac, that may be due to thicker coat that retarded the drug release from tablets (Table 6) . A marked increase in 5-FU release was observed in all cases of tablets with spores of probiotics as compared to tablets that do not contain spores at corresponding point of time. At the end of 24 hour studies all tablets with probiotics showed more that 90% drug release as compared to that of tablets without probiotics i.e. 83.64% (Table 5). It has been reported that concurrent administration of probiotics have shown positive impact and helped improving the condition of patients in treatment of colon cancer.[18] From the studies it is evident that addition probiotics are able to increase the amount of drug release both in coated and uncoated formulations. This is useful in conditions where the GIT micro flora is disturbed and in microflora absentia the drug release may not occur to the desired extent that is the purpose of colon targeting. The shellac coating controls the drug release and showed major portion (more than 80 %) released at target site i.e. colon.

CONCLUSION

From the present research work it may be concluded that matrix tablets coated with 10 % shellac solution that contain probiotics may be employed successfully for colon specific delivery to ensure maximum drug release at colon even in patients with disturbed gastrointestinal microflora. However, *in vivo* studies are required to comment more in this aspect.

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