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Research Article

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In Vitro Cytotoxicity Analysis of 5-Fluorouracil Loaded Guar Gum Microspheres on HT-29 Colon Cancer Cell Line

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ABSTRACT
The present investigation was aimed to develop a new formulation containing chemically cross-linked guar gum microspheres loaded with 5-fluorouracil for targeting of colorectal cancer. The emulsification polymerization method involving the dispersion of aqueous phase of guar gum in castor oil was used to prepare spherical microspheres. Cytotoxicity analysis on HT-29 human colon cancer cell lines indicated that 5-FU loaded guar gum microspheres leads to sustained releases of drug and thus delayed apoptosis over a long period of time. In this way, 5-fluorouracil loaded guar gum microspheres have shown promising results in the management of colorectal cancer, warranting thorough *in vivo* study for scale up technology.

Keywords: Microspheres, 5-fluorouracil, HT-29 human colon cancer cell lines.

INTRODUCTION

5-FU is an anticancer agent, interferes with nucleic acid synthesis and inhibits DNA synthesis. Because of its incomplete and erratic oral bioavailability, 5-FU is commonly administered intravenously. However, patients prefer oral rather than intravenous therapy, with oral treatment potentially more convenient and easy. [1] Site-specific delivery of 5-FU may reduce the systemic side effects and provide effective and safe therapy of colorectal cancer that may reduce the dose and duration of therapy when compared with the conventional treatment. [2] The coating of pH-sensitive polymers to the tablets, capsules or pellets provides sustained release along with protection of the active drug from gastric fluid. [3-4] Polysaccharides, the polymers of monosaccharides, retain their integrity in the upper gastrointestinal tract because they are resistant to the digestive action of gastrointestinal enzymes. The polysaccharide (guar gum) obtained from the seeds of *Cyamposis tetragonolobus* consists of linear chains of (1→4)β-D-manopyranosy1 units with ∝-D-galactopyranosy1 units attached by (1→6) linkages. [5] It is hydrophilic in nature and swells in cold water forming viscous colloidal dispersions or sols. This gelling property retards release of the drug from the dosage form and it is susceptible to degradation in the colonic environment. In the present investigation, a colon specific drug release systems was developed for 5-fluorouracil using a natural polysaccharide, guar gum and its cytotoxicity analysis was carried out on HT-29 human colon cancer cell lines.

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MATERIALS AND METHODS

Materials

M/s, Shalaks Pharmaceuticals Private Limited (New Delhi, India) generously supplied 5-Fluorouracil as gift sample. Guar gum was purchased from Central Drug House, New Delhi, India. Span 80, Tween 80 and Antifoam A were procured from Sigma Chemicals, USA. Glutaraldehyde (25% aqueous solution) was procured from S.D Fine Chemicals, Mumbai, India. All other solvents and reagents were of analytical grade.

Preparation of guar gum microspheres

Guar gum microspheres were prepared by emulsion polymerization technique. [6] The guar gum dispersion (2-10 % w/v) was prepared by mixing guar gum with Tween 80 (0.2% w/w) followed by the addition of aqueous solution of drug. Concentrated sulphuric acid (0.2 ml) was added and the aqueous phase was poured into castor oil (kept at 60°C) containing Span 80 (0.5 %w/w) and antifoam A (0.1 % w/w). The system was kept under agitation using stirrer (Eltek, Mumbai, India) at various speeds (2000 to 4000 rpm). Thereafter, glutaraldehyde (2 ml) was added under stirring

condition for 1 h. The microspheres were collected by centrifugation (Remi, Mumbai, India) washed 3-4 times with 15 ml isopropyl alcohol to remove traces of oil and dried in a vacuum desiccator for 48 h.

Characterization of 5-Fluorouracil loaded guar gum microspheres

Earlier work characterized the 5-Fluorouracil loaded guar gum microspheres for particle size, entrapment efficiency, *in vitro* release and drug-polymer interaction [7] and the optimized formulation has been used to study the in vitro cytotoxicity on HT-29 colon cancer cell lines.

***In vitro* cytotoxicity analysis of non-embedded and embedded 5-Fluorouracil on HT-29 human colon cancer cell lines**

The HT-29 human colon cancer cell lines were purchased from National cell lines facility, Pune and cultured in DMEM (Dulbecco's Modified Eagle Medium) medium supplemented with 10 % fetal bovine serum (FBS) at 37°C in a 5 % CO_2 atmosphere. To examine the effects of non-embedded 5-FU and embedded (guar gum bearing 5-FU), the cells were treated with 150 mM, 100 mM, 50 mM and similar concentrations of embedded 5-FU.

MTT assay

The MTT [3, (4, 5-Demethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay was performed as per standard protocol. The HT-29 human colon cancer cells were cultured in 24 well plates at a density of 5x 10^4 cells per well**.** The cells were treated with varying concentrations of 5-FU and embedded 5-FU. After 48 h, the cells were washed and treated with MTT. Plates were incubated in dark for 4 h, and the absorbance was measured at 570 nm using a microtitre plate reader. To determine the cell viability, percent viability was calculated:

[(absorbance of drug-treated) sample/ (control absorbance)] x 100.

Table 1: Percent viability of 5-FU in free and encapsulated form after 24 h

Sr. No.	Concentration	Free 5-FU	Encapsulated 5-FU
1.	150 mM	49.87 %	90.32 %
2.	100 mM	94.99 %	99.96 %
3.	50 mM	96.52 %	99.99 %

Table 2: Percent viability of 5-FU in free and encapsulated form after 48 h

Sr. No.	Concentration	Free 5-FU	Encapsulated 5-FU
1.	150 mM	26.78 %	65.28 %
2.	100 mM	42.22 %	85.24 %
3.	50 mM	79.15 %	92.18 %

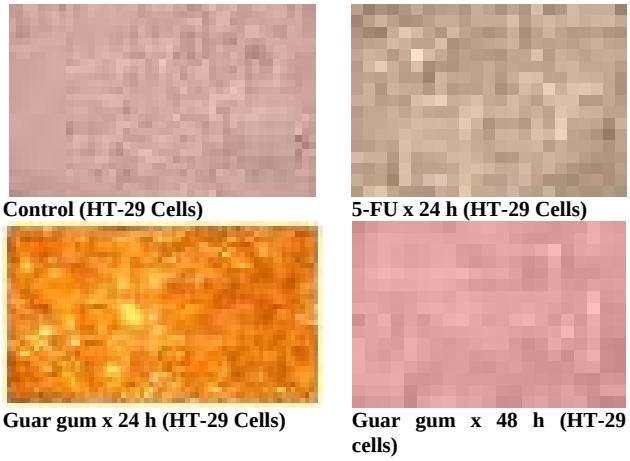
Table 3: Percent viability of 5-FU in free and encapsulated form after 72 h

Sr. No.	Concentration	Free 5-FU	Encapsulated 5-FU
1.	150 mM	15.70 %	49.36 %
2.	100 mM	18.38 %	47.26 %
3.	50 mM	22.68 %	40.31 %

RESULTS AND DISCUSSION

In the present investigation, guar gum microspheres crosslinked with glutaraldehyde were prepared for colon-targeting of 5-FU. The acidic medium required for cross-linking was imparted by the addition of concentrated sulphuric acid. The effect of various process variables such as stirring speed, glutaraldehyde treatment and temperature was studied in order to optimize the formulation. It was observed that these variables considerably influenced the shape, size as well as the entrapment efficiency of formulations. [7] The cytotoxicity analysis of 5-FU loaded guar gum microspheres was carried out on HIT-29 human colon cancer cell lines. Results indicated that when the HT-29 cells were treated with free 5-FU for 24 h (Fig. 1), it causes immediate apoptosis at different concentrations (50 mM to 150 mM; Table 1) in cancer cells in comparison of embedded 5-FU (5-FU loaded guar gum microspheres), which showed maximum 99.99% cell viability at 50 mM. This might be attributed to gel barrier of guar gum, which retard the release of 5-FU from guar gum microspheres. Moreover, it was observed that 72 h treatment (Table 3) of embedded 5-FU enhanced the apoptosis process and decreases the cell viability to 65.28 % at 150mM concentration.

The efficacy of the guar gum was evaluated for colon targeted drug delivery by fabricating it into microspheres. The studies demonstrated the ability of the guar gum as a carrier for targeted drug delivery to colon. Moreover, in vitro cytotoxicity analysis on HT-29 colon cancer cell lines demonstrated that 5-FU loaded guar gum microspheres possess the potential to delayed the apoptosis for long time.



Control (HT-29 Cells) **5-FU x 24 h (HT-29 Cells)**

Guar gum x 24 h (HT-29 Cells) **Guar gum x 48 h (HT-29 cells)**

Fig. 1 Photomicrographs of HT-29 human colon cancer cell lines treated with free 5-FU and 5-FU loaded guar gum microspheres

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REFERENCES

1. Heidelberger, C, Chaudhuri, NK, Danneburg P. Fluorinated pyrimidine, A new class of tumor inhibitory compounds. Nature 1957; 179, 663-666.
2. Chourasia, MK, Jain SK. Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharm Sci 2003; 6: 33-66.
3. Wilding IR, Hardy JG, Sparrow RA, Davis SS, Duly PB, English JR. In vivo evaluation of enteric coated naproxan tablets using gamma scintigraphy. Pharm Res 1992; 1436-1441.
4. Khan MZ, Prebeg Z, Kurjakovic N. A pH dependent colon targeted oral drug delivery sytem using methacrylic acid copolymers I. Manipulation of drug releases using Eudragit L100-55and Eudragit S100 combinations. J Control Release 1999; 58: 215-222.
5. Lippold BH, Sutter BK, Lippold BC. Parameters controlling drug release from pellets coated with aqueous ethyl cellulose dispersions [J]. Int J Pharm 1989; 54: 15-25.
6. Choursia MK, Jain SK. Potential of guar gum microspheres for target specific drug release to colon [J]. J Drug Targeting 2004; 12 (7): 435-442.
7. Kaushik D, Sardana S, Mishra DN. 5-Fluorouracil loaded guar gum microspheres for colon delivery: Preparation, characterization, In vitro release and cytotoxicity evaluation. Acta Pharmaceutica Sinica [in press].