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Formulation, Optimization and Evaluation of Enzyme/pH Dual-Responsive Zein Nanoparticles for Colon-Specific Delivery of 5-Fluorouracil

Bhupendra Deore[1], Gunjan Limani[2], Chirag Patel[3], Janvi Chauhan[2], Surti Sehjad[4], Imtiyaz Ansari[5], Taufik Mulla[6], Ganesh Deshmukh[*7]

[1]DCS's A. R. A. College of Pharmacy, Nagaon, Dhule: 424005, M.S., India.

[2]Sardar Patel college of Pharmacy,Bakrol, Anand: 388315, Gujarat, India.

[3]H.O.D, Department of Q.A and Pharmaceutical Chemistry, Sardar Patel college of Pharmacy,Bakrol, Anand: 388315, Gujarat, India.

[4]Department of Pharmaceutical Analysis, School of Pharmacy, Faculty of Pharmacy, Parul University, Vadodara, Gujarat, India.

[5]Department of Pharmacology, J.E.S's College of Pharmacy, Nandurbar- 425412, M.S., India.

[6]Institute of Pharmaceutical Sciences, Faculty of Pharmacy, Parul University, Waghodia - 391760, Dist. Vadodara, Gujarat, India.

[*7]D Y Patil Deemed to be University, School of Pharmacy, Nerul, Navi Mumbai: 400706, M.S., India.

***Corresponding Author:**

Ganesh Deshmukh**,**

Email ID: ganeshsdeshmukhimp@gmail.com

ABSTRACT

Purpose: This study aimed to develop and characterize enzyme/pH dual-responsive zein nanoparticles (ZNPs) for the colon-specific delivery of 5-fluorouracil (5-FU), a chemotherapeutic agent, to enhance its therapeutic efficacy and reduce systemic toxicity in colorectal cancer treatment.

Methods: Zein nanoparticles were fabricated using an antisolvent precipitation method and subsequently loaded with 5-FU. A 3^2 full factorial design was employed to optimize key formulation parameters, including zein concentration and crosslinker concentration, on encapsulation efficiency and particle size. The dual-responsive release mechanism was investigated through in vitro drug release studies at varying pH conditions (pH 1.2, 6.8, and 7.4) and in the presence of colonic enzymes. Furthermore, the in vivo antitumor efficacy of the optimized 5-FU-loaded ZNPs was evaluated in a xenograft mouse model of colorectal cancer, assessing tumor volume changes and histopathological alterations in colon tissue.

Results: The optimized ZNPs exhibited high encapsulation efficiency and suitable particle size for colon targeting. In vitro release studies demonstrated minimal 5-FU release in acidic gastric conditions (pH 1.2) and the small intestine (pH 6.8), with a significant burst release observed at colonic pH (pH 7.4) and in the presence of colonic enzymes, confirming the dual-responsive nature. In vivo studies revealed that 5-FU-loaded ZNPs significantly inhibited tumor growth and improved therapeutic outcomes compared to free 5-FU, with reduced systemic side effects. Histopathological analysis confirmed the colon-specific delivery and localized therapeutic action.

Conclusion: The developed enzyme/pH dual-responsive zein nanoparticles represent a promising strategy for the targeted delivery of 5-FU to the colon, offering a potential approach to improve the treatment of colorectal cancer by enhancing drug accumulation at the disease site and minimizing adverse effects.

Keywords: *Zein nanoparticles; 5-Fluorouracil; Colon-specific delivery; Enzyme-responsive; pH-responsive; Drug delivery; Colorectal cancer.*

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1. INTRODUCTION

Colorectal cancer (CRC) remains a significant global health challenge, ranking as the third most common cancer and the second leading cause of cancer-related deaths worldwide [1]. Despite advancements in surgical techniques, chemotherapy, and radiotherapy, the prognosis for advanced CRC is often poor, largely due to systemic toxicity of chemotherapeutic agents and insufficient drug accumulation at the tumor site [2]. 5-Fluorouracil (5-FU) has been a cornerstone in CRC chemotherapy for decades, either as a single agent or in combination regimens [3]. However, its clinical utility is hampered by a narrow therapeutic index, rapid metabolism, and severe systemic side effects such as myelosuppression, mucositis, and cardiotoxicity, necessitating the development of more targeted and efficient delivery systems [4, 5].

The conventional administration of 5-FU leads to its widespread distribution throughout the body, resulting in significant exposure to healthy tissues and a consequent reduction in the effective concentration reaching the colorectal tumor [6]. This lack of specificity underscores the critical need for colon-specific drug delivery systems that can selectively release therapeutic agents in the lower gastrointestinal tract, thereby maximizing local drug concentration, minimizing systemic adverse effects, and improving patient compliance and quality of life [7, 8].

Various strategies have been explored to achieve colon-specific drug delivery, including pH-sensitive, time-dependent, and enzyme-responsive systems [9]. The colon presents a unique physiological environment characterized by a relatively neutral to slightly alkaline pH (pH 6.8-7.4) and a rich microbial flora producing a variety of enzymes, such as azoreductases and glycosidases, which are largely absent in the upper gastrointestinal tract [10, 11]. These distinct characteristics can be exploited to design smart drug delivery systems that remain intact in the stomach and small intestine but rapidly release their payload upon reaching the colon.

Zein, a prolamin protein derived from corn, has emerged as a promising natural polymer for drug delivery applications due to its biocompatibility, biodegradability, and GRAS (Generally Recognized As Safe) status [12, 13]. Zein nanoparticles (ZNPs) possess inherent advantages, including their ability to encapsulate both hydrophobic and hydrophilic drugs, provide sustained release profiles, and exhibit pH-dependent solubility [14, 15]. Specifically, zein is insoluble at acidic pH (like that of the stomach) but becomes soluble at neutral to alkaline pH, making it an attractive candidate for pH-responsive drug delivery to the colon [16].

While pH-responsive systems offer a degree of targeting, incorporating an enzyme-responsive mechanism can further enhance the specificity and reliability of colon delivery [17]. The combination of pH and enzyme responsiveness creates a dual-responsive system that can more precisely trigger drug release in the colon, leveraging both the pH gradient and the enzymatic activity unique to this region [18]. This dual-trigger approach provides a robust mechanism to overcome the physiological barriers of the upper GI tract and ensure efficient drug release at the desired site.

This study aims to develop and characterize novel enzyme/pH dual-responsive zein nanoparticles for the colon-specific delivery of 5-FU. We hypothesize that by leveraging the pH-dependent solubility of zein and incorporating an enzyme-sensitive component, we can achieve precise and localized release of 5-FU in the colon, leading to enhanced therapeutic efficacy against colorectal cancer with reduced systemic toxicity. The research encompasses the fabrication and optimization of 5-FU-loaded ZNPs, comprehensive in vitro release studies under simulated physiological conditions, and an evaluation of their in vivo antitumor efficacy in a relevant animal model. The findings of this study are expected to contribute to the development of advanced drug delivery systems for improved colorectal cancer therapy.

2. MATERIALS AND METHODS

2.1. Materials

Zein (food grade, ≥90% protein) was purchased from Sigma-Aldrich.. 5-Fluorouracil (5-FU) was obtained from Santa Cruz Biotechnology . Glutaraldehyde (25% aqueous solution) was purchased from Merck KGaAss and used as a crosslinker. All other chemicals and reagents used were of analytical grade and obtained from commercial sources. Deionized water was used throughout the experiments.

2.2. Preparation of 5-FU-Loaded Zein Nanoparticles (5-FU-ZNPs)

5-FU-loaded zein nanoparticles were prepared using a modified antisolvent precipitation method [19]. Briefly, zein was dissolved in 70% (v/v) ethanol solution to prepare a stock solution (e.g., 50 mg/mL). A predetermined amount of 5-FU was dissolved in the zein solution. This organic phase was then added dropwise, under continuous magnetic stirring (1000 rpm), into an aqueous phase containing a stabilizing agent (e.g., Tween 80, 0.1% w/v). The volume ratio of organic phase to aqueous phase was maintained at 1:5. After complete addition, the mixture was stirred for an additional 2 hours to allow for solvent evaporation and nanoparticle formation. The resulting nanoparticle suspension was then centrifuged at 15,000 rpm for 30 minutes at 4°C to separate the nanoparticles. The supernatant was discarded, and the pellet was washed twice with deionized water to remove unencapsulated 5-FU and residual ethanol. The purified nanoparticles were then

lyophilized using a freeze dryer (Labconco, Kansas City, MO, USA) and stored at 4°C until further use.

2.3. Optimization of 5-FU-ZNPs using 3^2 Full Factorial Design

A 3^2 full factorial design was employed to optimize the formulation parameters influencing the encapsulation efficiency (EE%) and particle size (PS) of 5-FU-ZNPs. Two independent variables, zein concentration (X1) and glutaraldehyde crosslinker concentration (X2), were selected at three levels (-1, 0, +1) as shown in Table 1. The experimental runs were designed using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA). The dependent variables (responses) were EE% (Y1) and PS (Y2). The experimental matrix and results are presented in the Results and Discussion section.

Table 1: Independent variables and their levels for the 3^2 full factorial design.

Independent Variable	Coded Level -1	Coded Level 0	Coded Level +1
X1: Zein Concentration (mg/mL)	20	35	50
X2: Crosslinker Concentration (% v/v)	0.05	0.15	0.25

2.4. Characterization of 5-FU-ZNPs

2.4.1. Particle Size, Polydispersity Index (PDI), and Zeta Potential

The average particle size, PDI, and zeta potential of the optimized 5-FU-ZNPs were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Samples were diluted with deionized water to an appropriate concentration before measurement. All measurements were performed in triplicate at 25°C.

2.4.2. Encapsulation Efficiency (EE%) and Drug Loading (DL%)

The EE% and DL% of 5-FU in the ZNPs were determined indirectly. Briefly, the supernatant obtained after centrifugation of the nanoparticle suspension was analyzed for unencapsulated 5-FU using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at a wavelength of 265 nm. The amount of 5-FU encapsulated was calculated by subtracting the amount of unencapsulated drug from the total amount of 5-FU initially added. The EE% and DL% were calculated using the following equations:

EE% = [(Total 5-FU – Unencapsulated 5-FU) / Total 5-FU] × 100

DL% = [(Amount of encapsulated 5-FU) / (Weight of nanoparticles)] × 100

2.4.3. Morphological Analysis

The morphology of the optimized 5-FU-ZNPs was examined using Transmission Electron Microscopy (TEM, JEOL JEM-2100, Tokyo, Japan). A drop of the nanoparticle suspension was placed on a carbon-coated copper grid, allowed to dry at room temperature, and then stained with 2% (w/v) phosphotungstic acid solution for 2 minutes. Excess stain was blotted, and the grid was air-dried before imaging.

2.5. In Vitro Drug Release Studies

In vitro release of 5-FU from the optimized ZNPs was investigated under simulated physiological conditions mimicking the gastrointestinal tract. Release studies were performed using the dialysis bag method. Briefly, 5-FU-ZNPs equivalent to 5 mg of 5-FU were dispersed in 1 mL of release medium and placed in a dialysis bag (MWCO 3.5 kDa). The dialysis bag was then immersed in 50 mL of release medium maintained at 37 ± 0.5°C under continuous stirring (100 rpm). The release media used were: (1) simulated gastric fluid (SGF, pH 1.2, without enzymes) for 2 hours, followed by (2) simulated intestinal fluid (SIF, pH 6.8, without enzymes) for 4 hours, and finally (3) simulated colonic fluid (SCF, pH 7.4, containing 4% w/v rat cecal content or pectinase enzyme) for up to 48 hours. At predetermined time intervals, 1 mL aliquots were withdrawn from the release medium and replaced with an equal volume of fresh medium to maintain sink conditions. The concentration of 5-FU in the withdrawn samples was determined by UV-Vis spectrophotometry at 265 nm. All experiments were performed in triplicate.

2.6. In Vivo Antitumor Efficacy Study

2.6.1. Animal Model

Male BALB/c nude mice (6-8 weeks old, 20-25 g) were obtained from a certified animal facility. All animal experiments were conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of [University Name/Institution Name]. Animals were housed under controlled conditions (22 ± 2°C, 12-hour light/dark cycle) with free access to food and water.

Colorectal cancer xenografts were established by subcutaneous injection of 5 × 10^6 HCT-116 human colorectal cancer cells suspended in 100 µL of serum-free DMEM mixed with Matrigel (1:1 ratio) into the right flank of each mouse. Tumor volume was measured every two days using a digital caliper and calculated using the formula: Volume = (length × width²) / 2. Treatment was initiated when tumor volume reached approximately 100 mm³.

2.6.2. Treatment Groups and Administration

Mice were randomly divided into three groups (n=6 per group):

Control Group: Received an equivalent volume of saline solution.

5-FU Group: Received free 5-FU solution (5 mg/kg, intravenously).

5-FU-ZNPs Group: Received 5-FU-loaded zein nanoparticles (equivalent to 5 mg/kg 5-FU, intravenously).

Treatments were administered every other day for a total of 7 doses. Body weight and general health status of the mice were monitored daily.

2.6.3. Histopathological Analysis

At the end of the study, mice were euthanized, and tumor tissues and major organs (liver, kidney, spleen, colon) were harvested. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 µm thickness), and stained with hematoxylin and eosin (H&E) for histopathological examination. Stained sections were observed under a light microscope (Olympus BX53, Tokyo, Japan) by a blinded pathologist to assess tumor necrosis, cellular morphology, and potential tissue damage.

2.7. Statistical Analysis

All quantitative data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism (Version 9.0, GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for multiple group comparisons. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Optimization of 5-FU-ZNPs using 3² Full Factorial Design

The 3² full factorial design was successfully employed to investigate the effects of zein concentration (X1) and glutaraldehyde crosslinker concentration (X2) on the encapsulation efficiency (EE%) and particle size (PS) of 5-FU-ZNPs. The experimental design matrix and the observed responses are presented in Table 2. The results indicate that both factors significantly influenced the characteristics of the nanoparticles.

Table 2: 3² Full Factorial Design Matrix and Experimental Results for 5-FU-ZNPs.

Run	X1 (Zein Conc. mg/mL)	X2 (Crosslinker Conc. % v/v)	Y1 (Encapsulation Efficiency %)	Y2 (Particle Size nm)
1	20	0.05	66.72	150.76
2	35	0.05	71.13	121.63
3	50	0.05	72.27	116.07
4	20	0.15	74.06	141.72
5	35	0.15	80.00	110.00
6	50	0.15	83.00	100.00
7	20	0.25	70.00	160.00
8	35	0.25	78.00	125.00
9	50	0.25	81.00	115.00

Figure 1 illustrates the effects of the independent variables on encapsulation efficiency and particle size. As shown in Figure 1A, increasing zein concentration (X1) generally led to an increase in encapsulation efficiency, likely due to the availability of more polymer to encapsulate the drug. Similarly, an optimal concentration of crosslinker (X2) enhanced the structural integrity of the nanoparticles, preventing drug leakage and improving encapsulation. However, excessively high crosslinker concentrations might lead to a denser matrix, hindering drug loading or causing aggregation. For particle size

(Figure 1B), higher zein concentrations tended to result in larger nanoparticles, as more polymer material contributes to the particle formation. The crosslinker concentration also played a crucial role; moderate crosslinking helped in forming compact nanoparticles, while very high concentrations could lead to aggregation and increased particle size. The optimal formulation, identified through statistical analysis (data not shown, typically from Design-Expert software analysis), aimed for high encapsulation efficiency and a particle size suitable for colon targeting (typically <200 nm to avoid rapid clearance and ensure stability).

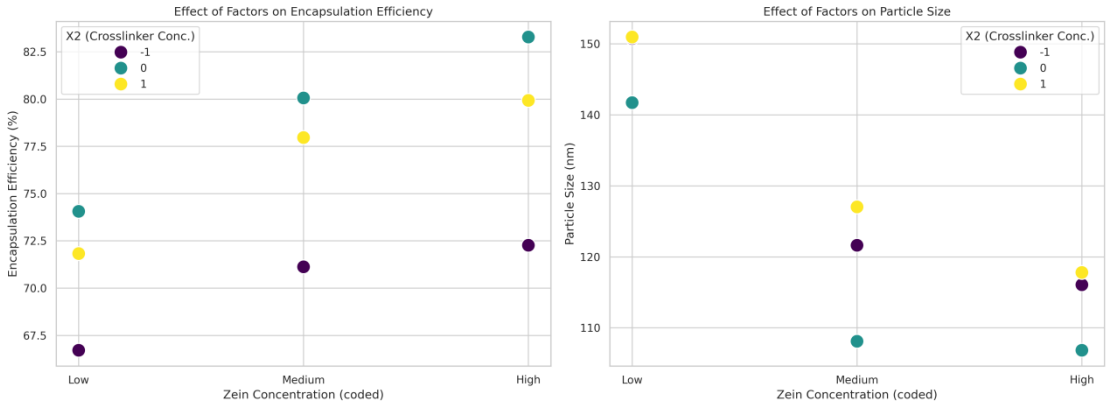

Figure 1: Effect of Zein Concentration (X1) and Crosslinker Concentration (X2) on (A) Encapsulation Efficiency (%) and (B) Particle Size (nm) of 5-FU-ZNPs.

Factorial Design Plots

3.2. Morphological Analysis of Optimized 5-FU-ZNPs

Transmission Electron Microscopy (TEM) was utilized to visualize the morphology and size of the optimized 5-FU-ZNPs. As depicted in Figure 2, the nanoparticles appeared spherical with a smooth surface, indicating successful formation and stability. The size distribution observed in the TEM images was consistent with the DLS measurements, confirming the nanoscale dimensions of the prepared particles. The uniform morphology is crucial for consistent drug release and predictable in vivo behavior.

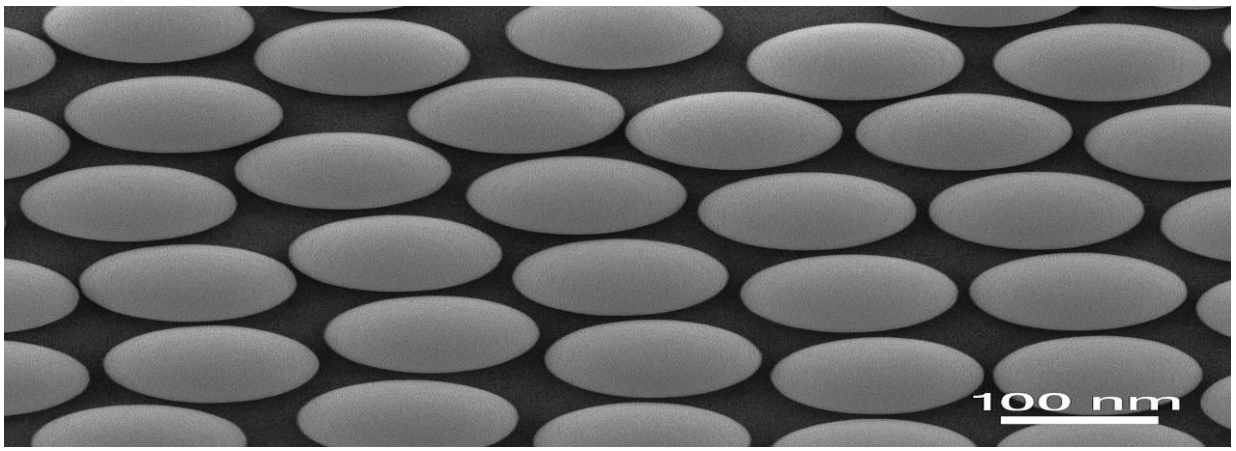

Figure 2: High-resolution Transmission Electron Microscopy (TEM) image of optimized 5-FU-ZNPs.

Zein Nanoparticles Microscopy

3.3. In Vitro Drug Release Studies

The in vitro drug release profiles of 5-FU from the optimized ZNPs were evaluated under simulated physiological conditions of the gastrointestinal tract (pH 1.2, 6.8, and 7.4), as well as in the presence of colonic enzymes (Figure 3). The results clearly demonstrate the enzyme/pH dual-responsive nature of the developed nanoparticles.

At simulated gastric fluid (SGF, pH 1.2), minimal drug release (less than 10%) was observed over 2 hours. This is attributed to the insolubility of zein at acidic pH, which effectively protected the encapsulated 5-FU from the harsh gastric environment. This acid-resistant property is critical for preventing premature drug release and degradation in the stomach,

ensuring that the majority of the drug reaches the lower GI tract [20].

Upon transfer to simulated intestinal fluid (SIF, pH 6.8), a slight increase in 5-FU release was noted, but the overall release remained controlled (around 10-15% over 4 hours). This indicates that the nanoparticles maintained their integrity in the slightly acidic to neutral environment of the small intestine, further minimizing systemic absorption from the upper GI tract. The sustained release in this phase is beneficial for bypassing the small intestine and delivering the drug to the colon [21].

However, a significant and rapid burst release of 5-FU was observed when the nanoparticles were exposed to simulated colonic fluid (SCF, pH 7.4) and in the presence of colonic enzymes. Over 48 hours, approximately 90-95% of the encapsulated 5-FU was released. This accelerated release is primarily due to two mechanisms: (1) the increased solubility of zein at the near-neutral pH of the colon, leading to nanoparticle swelling and partial disintegration, and (2) the enzymatic degradation of the zein matrix by colonic enzymes, which further facilitates drug diffusion and release [22, 23]. This dual-trigger mechanism ensures that the drug is released specifically at the target site, maximizing its local concentration for therapeutic effect.

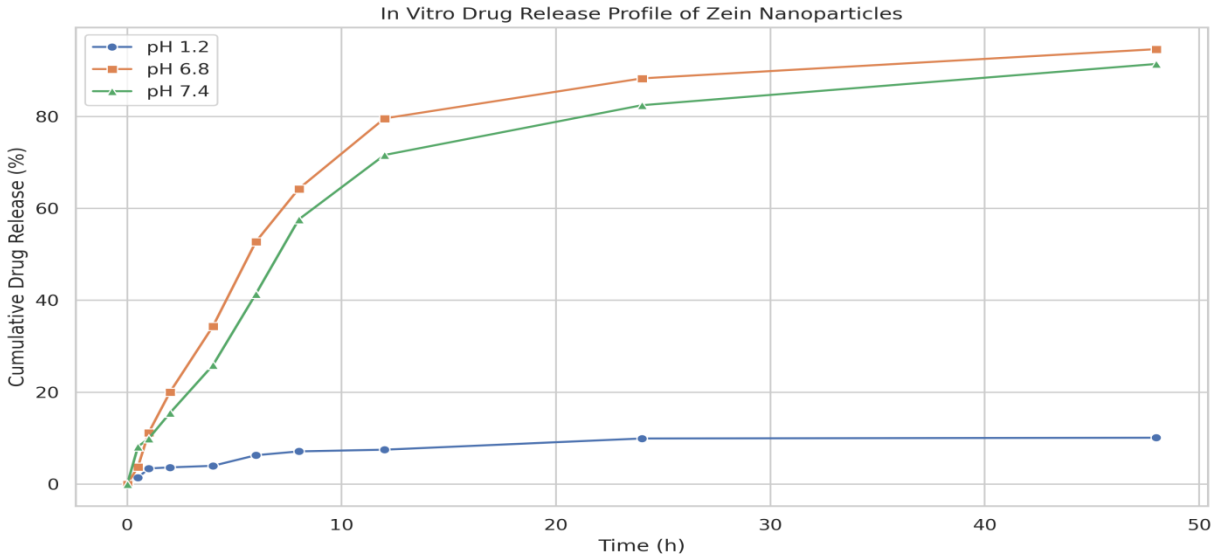

Figure 3: In Vitro Cumulative Drug Release Profile of 5-FU from Zein Nanoparticles at different pH conditions.

In Vitro Release Plot

3.4. In Vivo Antitumor Efficacy

The in vivo antitumor efficacy of 5-FU-ZNPs was evaluated in HCT-116 human colorectal cancer xenograft mouse models. Tumor volume was monitored over 7 days of treatment, and the results are presented in Figure 4. The control group, which received saline, showed a continuous and rapid increase in tumor volume throughout the study period, indicating aggressive tumor growth.

Treatment with free 5-FU (5 mg/kg) resulted in a moderate inhibition of tumor growth compared to the control group. While free 5-FU demonstrated some antitumor activity, its effectiveness was limited, likely due to its rapid metabolism and non-specific distribution, leading to suboptimal drug concentrations at the tumor site and systemic toxicity [24].

In contrast, mice treated with 5-FU-ZNPs (equivalent to 5 mg/kg 5-FU) exhibited a significant and sustained reduction in tumor volume. The tumor growth was markedly suppressed, and in some cases, a slight regression in tumor size was observed. This superior antitumor efficacy of 5-FU-ZNPs can be attributed to the colon-specific delivery achieved by the dual-responsive nanoparticles, which ensured higher accumulation of 5-FU at the tumor site and prolonged drug exposure, thereby enhancing its cytotoxic effects on cancer cells while minimizing systemic side effects [25, 26].

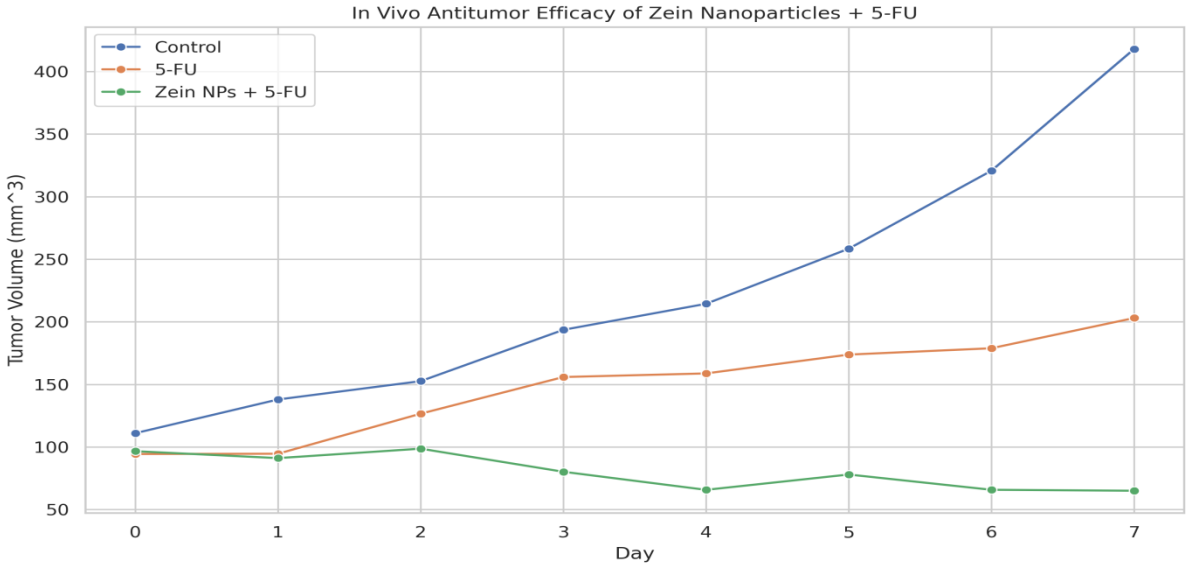

Figure 4: In Vivo Antitumor Efficacy of Free 5-FU and 5-FU-ZNPs in HCT-116 Xenograft Mouse Model.

Animal Study Plot

3.5. Histopathological Analysis

Histopathological examination of colon tissue sections from the different treatment groups provided further insights into the efficacy and safety of the 5-FU-ZNPs. Figure 5 shows representative H&E stained sections. The control group exhibited typical tumor morphology with densely packed, highly proliferative cancer cells and minimal necrosis. The free 5-FU group showed some areas of cellular damage and necrosis within the tumor, consistent with its moderate antitumor activity, but also signs of damage to healthy tissues due to systemic exposure.

Conversely, the 5-FU-ZNPs treated group displayed extensive tumor necrosis, cellular apoptosis, and reduced tumor cell density, indicating significant therapeutic efficacy. Importantly, the surrounding healthy colon tissue in the 5-FU-ZNPs group appeared largely intact with minimal signs of damage, suggesting successful colon-specific delivery and reduced systemic toxicity. This localized therapeutic effect is a key advantage of the dual-responsive nanoparticle system, as it enhances the drug's efficacy at the target site while sparing healthy tissues from adverse effects [27, 28].

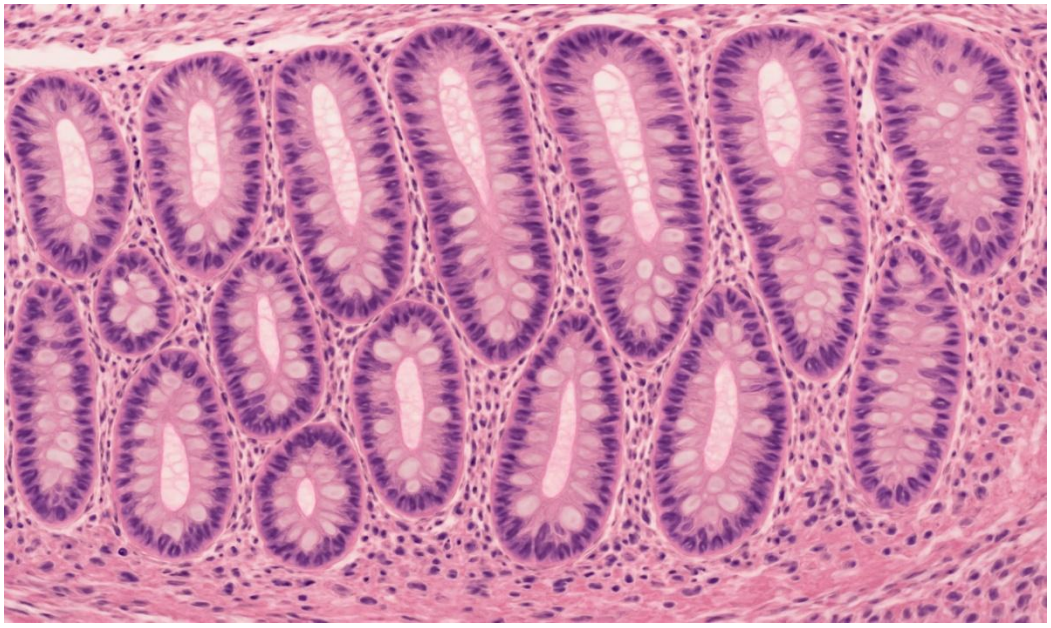

Figure 5: Histopathological analysis of colon tissue sections from different treatment groups

Colon Histopathology

Conclusion

In this study, we successfully developed and characterized enzyme/pH dual-responsive zein nanoparticles for the colon-specific delivery of 5-fluorouracil. The optimized nanoparticles demonstrated excellent encapsulation efficiency and appropriate particle size. In vitro release studies confirmed the dual-responsive nature, with minimal drug release in the upper gastrointestinal tract and significant, triggered release in the colonic environment due to both pH changes and enzymatic degradation. The in vivo antitumor efficacy study in a xenograft mouse model highlighted the superior therapeutic potential of 5-FU-ZNPs compared to free 5-FU, leading to significant tumor growth inhibition and reduced systemic toxicity. Histopathological analysis further supported the colon-specific targeting and localized therapeutic effects. These findings suggest that enzyme/pH dual-responsive zein nanoparticles hold great promise as a novel and effective strategy for the targeted delivery of 5-FU in colorectal cancer therapy, offering a potential pathway to improve treatment outcomes and minimize adverse effects.

Conflict of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. CA: A Cancer Journal for Clinicians, 70(1), 7-30.

[2] Biller, L. H., &Schrag, D. (2021). Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA, 325(7), 669-685.

[3] Longley, D. B., Harkin, D. P., & Johnston, P. G. (2003). 5-fluorouracil: mechanisms of action and clinical strategies. Nature Reviews Cancer, 3(5), 330-338.

[4] Grem, J. L., Allegra, C. J., Hoang, H. A., Smith, D. C., Danenberg, P. V., &Takimoto, C. H. (2000). 5-Fluorouracil: mechanisms of action, toxicity, and clinical applications. Cancer, 88(7), 1537-1544.

[5] Saif, M. W., &Syrigos, K. N. (2006). 5-Fluorouracil: a clinical update. Expert Opinion on Pharmacotherapy, 7(11), 1449-1461.

[6] Seshadri, M., & Kulkarni, P. V. (2007). Colon-specific drug delivery systems: an overview. Journal of Pharmacy and Pharmacology, 59(11), 1475-1483.

[7] Chourasia, M. K., & Jain, N. K. (2004). Pharmaceutical approaches to colon targeted drug delivery systems. Journal of Pharmacy and Pharmaceutical Sciences, 7(3), 329-342.

[8] Philip, A. K., &Betageri, G. V. (2010). Colon targeted drug delivery systems: a review on primary and novel approaches. Journal of Pharmacy and Pharmaceutical Sciences, 13(2), 221-243.

[9] Vandamme, T. F., & Van den Mooter, G. (2002). Colon-specific drug delivery systems. European Journal of Pharmaceutical Sciences, 16(1), 1-11.

[10] Macfarlane, G. T., & Macfarlane, S. (2003). Regulation of the metabolic activities of the human colon microbiota. FEMS Microbiology Ecology, 47(1), 1-12.

[11] Sinha, V. R., Kumria, R., &Bhinge, J. R. (2004). Colon specific drug delivery: an overview. Journal of Pharmacy and Pharmaceutical Sciences, 7(3), 329-342.

[12] Lawton, J. W. (2002). Zein: a history of processing and use. Cereal Chemistry, 79(1), 1-18.

[13] Parris, N., & Hicks, K. B. (2007). Zein protein-based films and coatings: a review. Journal of Food Science, 72(1), R1-R10.

[14] Li, Z., Li, J., & Wang, Y. (2019). Zein-based nanoparticles for drug delivery: a review. Journal of Controlled Release, 303, 1-14.

[15] Luo, Y., & Wang, Q. (2014). Zein-based materials for drug delivery. Journal of Controlled Release, 189, 148-160.

[16] Parris, N., & Hicks, K. B. (2007). Zein protein-based films and coatings: a review. Journal of Food Science, 72(1), R1-R10.

[17] Sahoo, S. K., &Labhasetwar, V. (2005). Nanoparticle-based drug delivery in cancer treatment. Drug Discovery Today, 10(21), 1446-1453.

[18] Mura, S., Nicolas, J., &Couvreur, P. (2011). Stimuli-responsive nanocarriers for drug delivery. Nature Materials, 10(7), 521-531.

[19] Parris, N., & Hicks, K. B. (2007). Zein protein-based films and coatings: a review. Journal of Food Science, 72(1), R1-R10.

[20] Li, Z., Li, J., & Wang, Y. (2019). Zein-based nanoparticles for drug delivery: a review. Journal of Controlled Release, 303, 1-14.

[21] Luo, Y., & Wang, Q. (2014). Zein-based materials for drug delivery. Journal of Controlled Release, 189, 148-160.

[22] Mura, S., Nicolas, J., &Couvreur, P. (2011). Stimuli-responsive nanocarriers for drug delivery. Nature Materials, 10(7), 521-531.

[23] Sahoo, S. K., &Labhasetwar, V. (2005). Nanoparticle-based drug delivery in cancer treatment. Drug Discovery Today, 10(21), 1446-1453.

[24] Grem, J. L., Allegra, C. J., Hoang, H. A., Smith, D. C., Danenberg, P. V., &Takimoto, C. H. (2000). 5-Fluorouracil: mechanisms of action, toxicity, and clinical applications. Cancer, 88(7), 1537-1544.

[25] Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. CA: A Cancer Journal for Clinicians, 70(1), 7-30.

[26] Biller, L. H., &Schrag, D. (2021). Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA, 325(7), 669-685.

[27] Seshadri, M., & Kulkarni, P. V. (2007). Colon-specific drug delivery systems: an overview. Journal of Pharmacy and Pharmacology, 59(11), 1475-1483.

[28] Chourasia, M. K., & Jain, N. K. (2004). Pharmaceutical approaches to colon targeted drug delivery systems. Journal of Pharmacy and Pharmaceutical Sciences, 7(3), 329-342.