Vol. (1), No (1), January-March 2024 10.21608/MERMJ.2024.272097.1012

Porous microparticles as a tool for minimizing 5-Fluorouracil induced intestinal mucositis

Ahmed U.Ali1[1]*, Mahmoud El Badry[2, 7] , Ahmed M Haredy [3], Heba A. Abou- Taleb4, Mohamed Gamal Thabit[5], Heba A. El-Din Mubarak[6], and Tahani H. El Faham[7]

.

1. Department of Pharmaceutics, Faculty of Pharmacy, Merit University.
2. Assiut International Center of Nanomedicine, Al-Rajhy Liver Hospital, Assiut University,.
3. Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Merit University,
4. Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Merit University,
5. Lecturer of pharmaceutical organic chemistry and medicinal chemistry – Faculty of pharmacy – Merit University.
6. Department of Histology, Faculty of Medicine, Assiut University.
7. Department of Pharmaceutics, Faculty of Pharmacy, Assiut University.

* Corresponding author.

ARTICLEINFO

Article history:
Received 22 February2024
Accepted 26 June 2024
Available online 22July 2024

Keywords:
5-Fluorouracil, oral administration, mucositis, and histological study..

Abstract

5 Fluorouracil (5-FU) is used primarily to treat solid malignant tumors and is administered via the intravenous route; however, this administration is accompanied by intestinal mucositis, represented by diarrhea. In this work, 5-FU microsponges (porous microparticles) were prepared to be taken orally to minimize the intestinal mucositis associated with the intravenous IV administration of 5-FU. 15 male albino rabbits were utilized to compare the degree of intestinal mucositis related to the intravenous administration to that of 5-FU microsponges capsule oral administration (at the same dose). The experiment was conducted for five consecutive days. Results showed that the mean villus height of the IV group measured about 260.0± 6.9 µm, which was considerably lower ($p<0.05$) than that of the Capsule group, which measured about 285.0± 9.3 µm; in addition, the mean depth of crypts of IV group measured about 110.0± 6.8 µm which is significant ($p<0.05$) lower than that of Capsule group which measured about 141.0± 7.8 µm which means that the degree of intestinal mucositis induced by the intravenous injection of 5-FU was significantly higher than that caused by that of oral administration of 5- FU microsponges formulation. By the way, on the fourth day, one rabbit of the IV group died, indicating the severity of the intestinal mucositis after the IV administration of 5-FU. The results showed that the oral administration of 5-FU microsponges is more safe than IV drug administration at the same dose.

Introduction

Because of its capacity to increase tumor-free status and survival rates, the antimetabolite chemotherapeutic agent (5-FU) is regarded as one of the most effective and widely used medications for the treatment of a variety of malignancies, including head, neck, breast, and gastrointestinal tract cancers [1, 2]. But because of its non-specific mode of action, the enterocytes lining the small intestine sustain collateral damage, which leads to the development of mucositis. In addition to histological features of reduced villus length and crypt disruption along with inflammation and ulceration of the intestinal mucosa, mucositis is characterized by excruciating pain, nausea, bloating, diarrhea, and loss of appetite. These symptoms compromise intestinal function [3]. Interestingly, mucositis significantly lowers the quality of life in patients subjected to chemotherapy regimens[4]; it most frequently affects the mucosa of the mouth and small intestine but can affect the entire gastrointestinal system. Additionally, between 50 and 80 percent of patients receiving 5-FU experience intestinal diarrhea, a significant adverse effect linked to the intravenous administration of this medication [1].

Microsponges are large-surface, porous polymeric microparticles. As free-flowing particles, they have a high surface area, better thermal, physical, and chemical stability, and many other benefits as a drug delivery mechanism [5] and are cost-effective. They also disperse throughout the

gastrointestinal tract (GIT), which results in a more consistent medication release, less discomfort, and prolonged retention.

Mucosal tissue damage brought on by inflammatory cell infiltration, pro-inflammatory cytokine release, and edema results in mucositis, which is defined as villus atrophy, disturbance of the intestinal absorptive surface, and crypt necrosis [6]. This syndrome develops as 5-FU exerts its functions in cells that are in the S phase of the cell cycle, inhibiting the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), therefore, interrupting cell proliferation [4, 7]. Consequently, oxidative stress is promoted, leading to cytotoxic effects on the cells. However, these effects are not restricted to cancer cells; the drug also acts on all proliferative cells, such as mucosal membrane cells [7]. Mucositis develops as 5-FU exerts its functions in cells in the S phase of the cell cycle, inhibiting the synthesis of DNA and RNA, therefore interrupting cell proliferation [4, 8]. Consequently, oxidative stress is promoted, leading to cytotoxic effects on the cells. However, these effects are not restricted to cancer cells; the drug also acts on all proliferative cells, such as mucosal membrane cells [8].

High performance liquid chromatography (HPLC) is widely used to determine 5- FU in plasma samples. HPLC may be normal-phase [9], which necessitates derivatization, Reverse-phase [9-13], ion-pair [14], and reversed-phase ion-pair (RP-IP). Reversed-phase ion-pair (RP-IP) was applied for the determination of 5-FU using ion pair chromatography where the mobile phase was a mixture of tetrabutylammonium hydroxide and methanol; the detection limit was 10.0 ng/ml[14]. RP- HPLC is widely used for the determination of 5-FU in plasma.

5- FU is commonly administered via the intravenous route, either by bolus or continuous infusion. Petitet al. found that there was a wide variation (3: 25) fold in the plasma concentrations of the drug among patients receiving 5-FU by continuous infusion for 24 hours [15] that explained this observation by pointing to the intra-individual differences in the patients' dihydropyrimidine dehydrogenase enzyme (DPD) levels. [16, 17].

Oral administration of 5-FU (alone) has been stopped because of the high intra-individual variations between patients after oral administration. It was found that the bioavailability of 5-FU varies from (0-80) % between patients when the drug is administered by the oral route [18, 19]. Naguib et al. attributed this phenomenon to the fact that as 5-FU has a pKa of 8.1, the acidic pH of the upper gastrointestinal tract would enhance the absorption of the drug[20]. DPD, an enzyme that plays a major role in the catabolism of the drug, is present throughout the gastrointestinal tract mucosa. In the liver, about 85% of the administered drug is catabolized as the enzyme (DPD) is highly expressed[21] and is responsible for the intra-individual variation in the bioavailability of the drug after oral administration[20].

However, the intravenous injection of 5-FU is associated with a number of side effects, including gastrointestinal issues (mucositis), which manifest as diarrhea, and afflict between 50 and 80 percent of patients [1]. As the medication is absorbed, the small intestinal epithelium is harmed, resulting in additional adverse responses. Intestinal villi atrophy and loss have also been noted [22, 23]. This phenomenon can result in systemic infection and sometimes death. Mucositis can be so severe that chemotherapy regimens must be restructured or, in some cases, discontinued, altering patient prognosis [4]. Taking these facts into consideration, the present investigation is directed to seek a safer route of administration to minimize the side effects of the drug. Our study is conducted to investigate the histological effects raised by the colon-targeted preparation containing the microsponges formulation of this drug in addition to comparing the degree of intestinal mucositis caused by intravenous IV administration of 5-FU and that caused by the capsules containing microsponges formulation of this drug using different histology markers, such as assessments of villus height, crypt depth, and disease severity.

Materials and methods

Materials

5-FU was bought from China's AvansCure Lifesciences Private Limited. Eudragit RS100 (ERS-100) was obtained from Röhm, Germany was obtained from Alfa Aesar, Germany. Triethyl citrate and tribasic sodium phosphate were obtained from Oxford Laboratory Chemicals, India. Methylene chloride, Polyethylene glycol (PEG) (M. Wt 4000) from El- Nasr Company, Egypt.

Animals

In this investigation, fifteen male New Zealand rabbits in good health, weighing 2.0 ± 0.1 kg, were employed. The study was carried out in compliance with the normal operating procedures of the Assiut University's Committee on Animal Care, Assiut, Egypt. Twelve hours before the trial began; the animals were allowed unlimited access to water while fasting.

Methods

Construction of 5-FU calibration curve in distilled water, 0.1N HCL, and phosphate buffer

A stock solution I of 5-FU was prepared by dissolving 50.0 mg of 5-FU in 100.0 ml distilled water to give a concentration of 0.5 mg/ml. Then, 5.0 ml of stock solution I was diluted to 25.0 ml with distilled water to provide a concentration of 100.0µg/ml (stock solution II). Solutions of different concentrations in the 4.0-16.0µg/ml range were prepared by serial dilution of (stock solution II) using distilled water. The same procedures were performed in 0.1N HCL and phosphate buffer of pH 6.8, respectively. The absorbances were determined spectrophotometrically at the predetermined λ_{max}266 nm[24]against a similarly treated blank. The experiment was conducted in triplicate, and the average spectrophotometric absorbances were plotted against the drug concentrations (µg/ml) to construct the standard calibration curve of 5-FU.

Similar procedures were performed at the same concentrations and dilutions in 0.1N HCL and a mixture of (0.1 n HCL and tribasic sodium phosphate) at a pH of 6.8.

Microsponges preparation

The quasi-emulsion solvent diffusion method[25] was utilized to prepare microsponges enclosing 5- FU. To prepare the inner phase, ERS-100 was dissolved in methylene chloride, and TEC was added as a plasticizer. 5- FU was then added to the solution and was dispersed using ultrasonication for 300 seconds at 35 ˚C. This dispersion was then poured into the PEG 4000 solution. After a specific stirring time, the product was filtered and washed to obtain clear microsponges. The microsponges were then dried in the open air for 12 h at 25˚C to get the product.

Histological assessment of drug-induced intestinal mucositis

After the preparation Animals were subdivided into three groups; each one consisted of 5 rabbits (*n=3*). The first (control) group was kept as a control; the second group IV group was injected with a single intravenous IV bolus dose of 5- FU through the marginal ear vein. The third group (capsule group) received capsules containing the formulation of 5-FU microsponges. The experiment was conducted for 5 consecutive days. The protocols and experiment were done by virtue of the standards of the ethical committee of the Faculty of Pharmacy, Assiut University, Assiut, Egypt. The dose of 5-FU in each route was adjusted to be 5 mg/kg body weight and given once daily, regardless of the route of administration. The IV group, which received intravenous injection 1, died on the fourth day. The animals were killed by an overabundance of chloroform fumes at the end of the experiment. A mid-line incision was made to open their abdomens, and the jejunum was cut apart from the surrounding viscera and removed. It was then cleaned with physiological saline solution and submerged in freshly made, preserved 10.0% neutral formalin solution. After trimming, cleaning, and dehydration in increasing alcohol grades, the fixed specimens were put back together. Following xylene clearing, the specimens were embedded in paraffin, sectioned at 4.0-6.0 µm thickness, stained with PAS and Hx & E, and mounted in DPX for light microscopy examination [26] histological parameters (disease severity, crypt depth, no goblet cells over the villi, lymphocytic infiltration, and villus height measurements). Small intestinal villus height and crypt depth were measured in the jejunum (25 villi and 25 crypts per section) using a light microscope (Olympus BH-2, Tokyo, Japan) and digital camera (Sony, Tokyo, Japan) using analysis imaging software (Version 5.1, Olympus, Tokyo, Japan) and were quantified.

Results & discussion

Calibration curves of 5-FU were obtained by plotting absorbance versus 5-FU concentrations. (Figs. 1, 2 and 3) shows the standard calibration curve of 5-FU in distilled water, 0.1N HCL, and phosphate buffer of pH 6.8, respectively. The standard calibration curve was linear over the 4 -16 µg/ml concentration range with a correlation coefficient (R^2) of 0.9995.

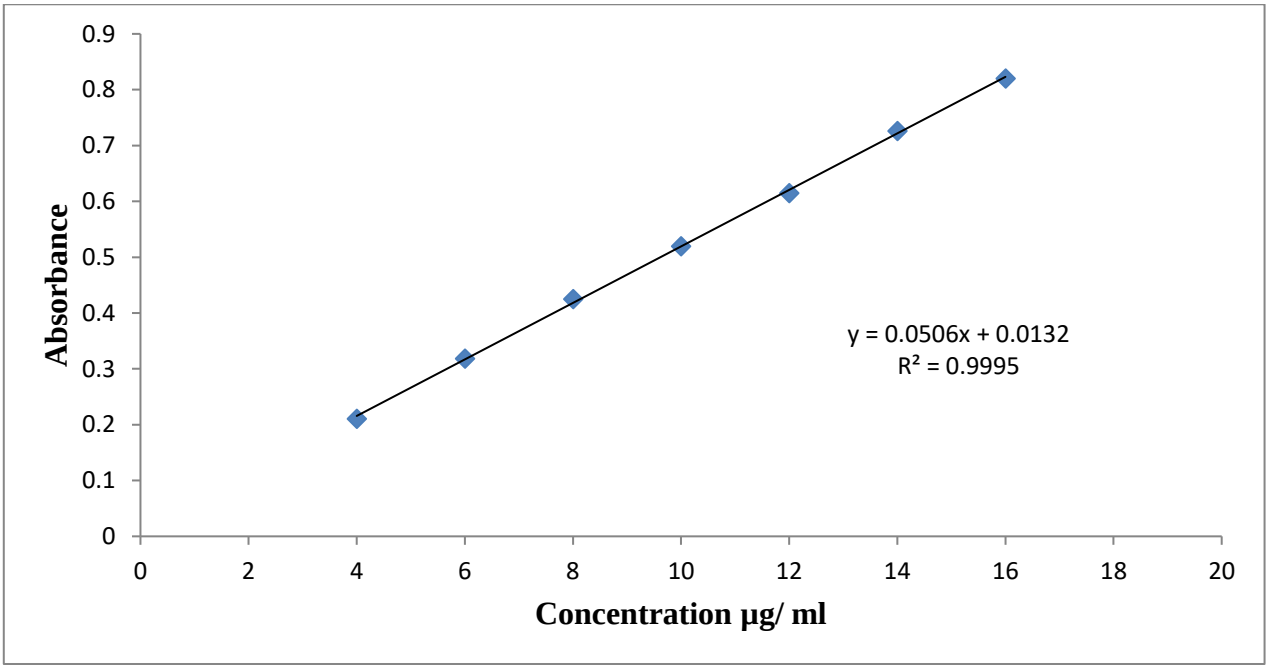

Fig. (1): Standard Calibration Curve of 5- FU in distilled water.

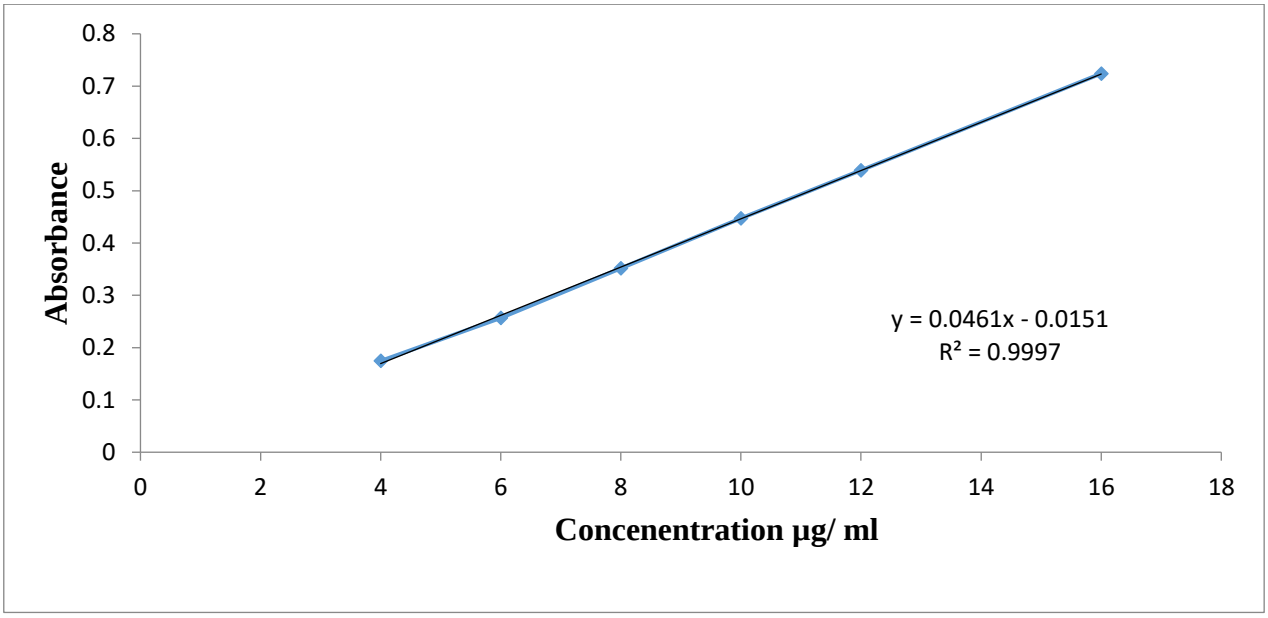

Fig. (2): Standard Calibration Curve of 5- FU in 0.1 N HCL.

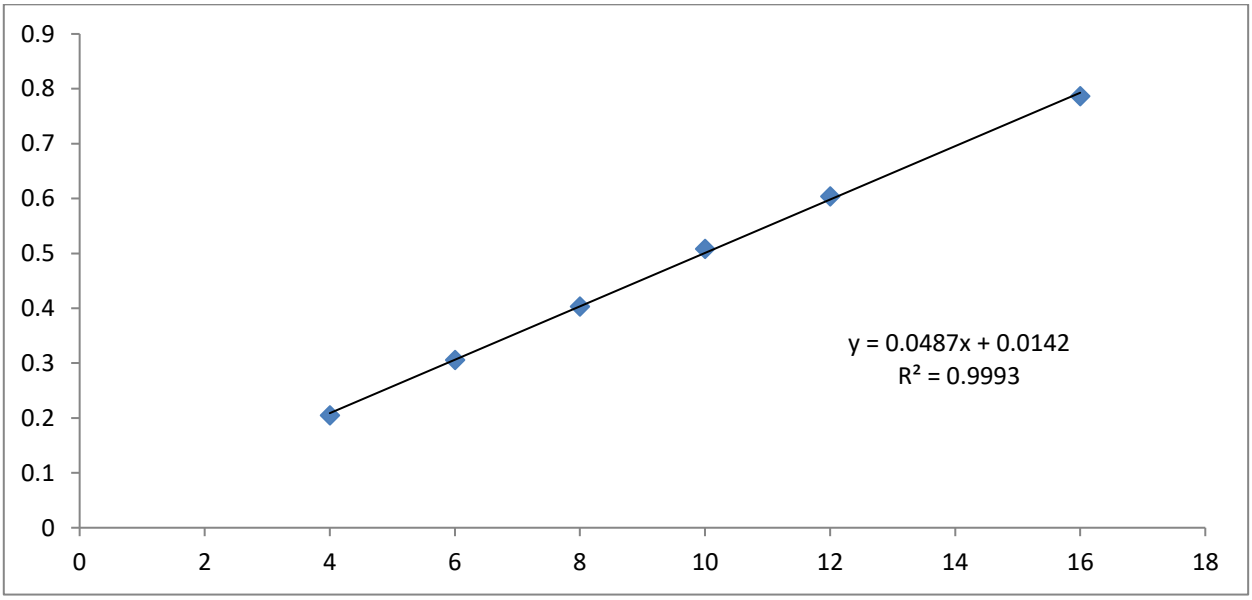

Fig. (3): Standard Calibration Curve of 5- FU in Phosphate buffer pH 6.8.

Characterization of prepared microsponges

The particle size of 5-FU microsponges formulation approached 160.44± 2.54 µm in diameter, the product yield was about 71.00±0.36%, the % encapsulation efficiency was found to be 78.61±01.76, and the % cumulative drug released from microsponges approached 78.85±0.36% after 24 hrs.

Histological study

After developing 5-FU microsponges formulation, it was packed into HPMC acid-resistant capsules and used for histological study. Light microscopical examination of the jejunum of the control group (Fig. 4a & b) revealed that the wall of the jejunum is formed of four coats, namely, mucosa, submucosa, muscular, and serosa. The innermost layer's mucosa is projected into the lumen with leaf-like or slender-shaped villi and shows simple tubular, less coiled intestinal glands in the underlying lamina propria. The villus measured about 310.0± 8.4 µm in height and was covered with a continuous acidophilic brush border epithelium. The epithelial covering showed columnar and goblet cells. However, the goblet cells had an apical expanded vacuolated part and a basal slender part containing a small compressed vertical nucleus. Some intraepithelial lymphocytes (about 8.0± 2.2 cells per 1000 μm^2) were observed in the covering epithelium of the villi at various levels from the basement membrane. This

basement membrane was intact and appeared firmly fitted into the underlying connective tissue of the core. The connective tissue core of intestinal villi showed blood capillaries, lymphatic capillaries, and some mononuclear cell infiltration, about 14.0± 2.9 cells per 1000 µm^2. The lamina propria below the villi is composed of dense connective tissue containing several lymphocytic infiltrations and appears packed with simple tubular glands, which open between the bases of the villi. Such glands showed a narrow lumen and lined with cubical to columnar cells together with mucous goblet cells. The goblet cells had roundish apical vacuolated cytoplasm and basal spherical nuclei near the basement membrane. The number of goblet cells per villus was about 28± 2.4.

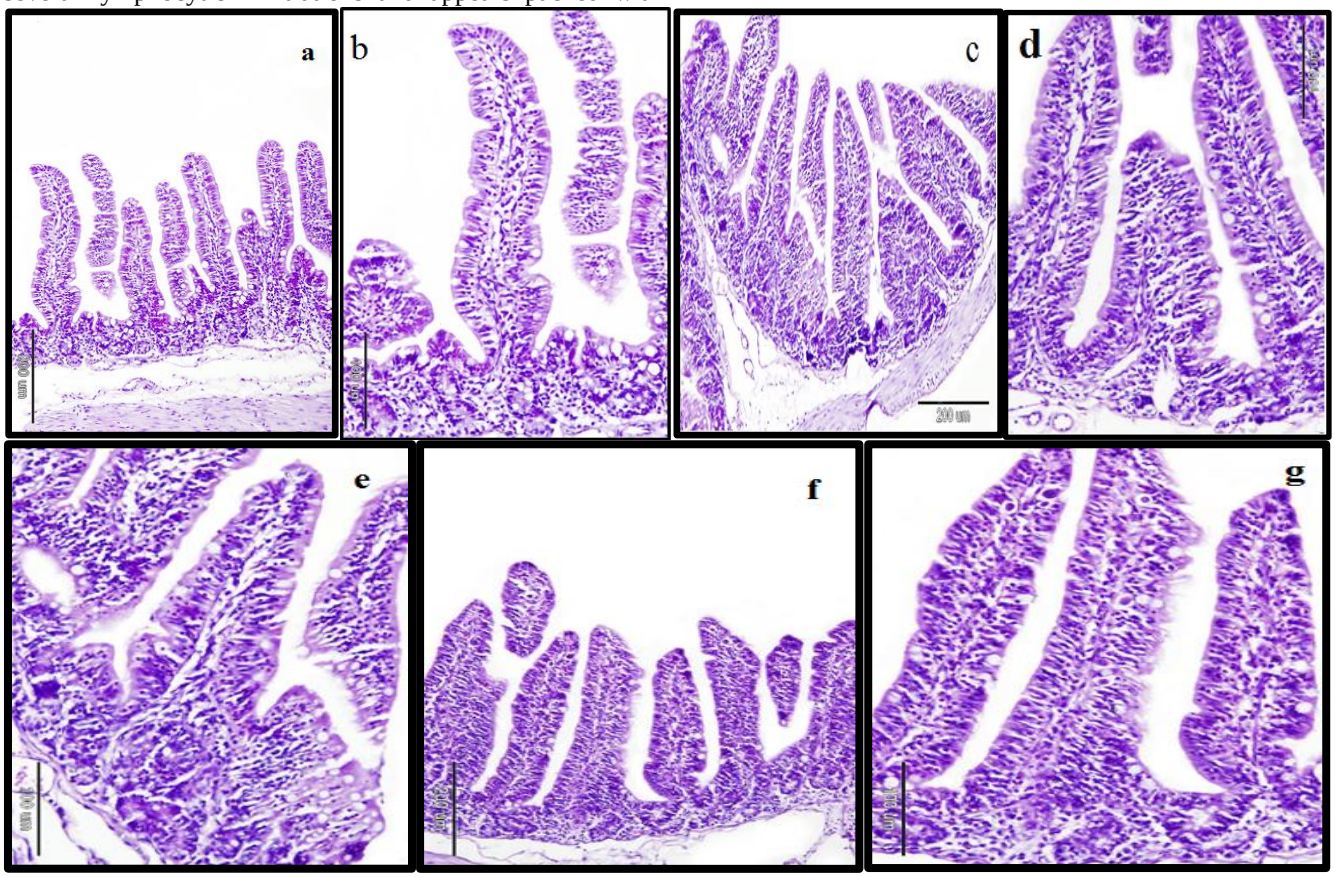

Fig. 4: Histological findings of rabbit jejunum **a**& **b** (control) group, **c**, **d,** and **e** (IV) group, and **f**& **g** (capsule) group.

The present investigation of the jejunum of IV (Fig.1 c-e) group showed moderate distortion of its covering epithelium, interrupted brush border, and appearance of intercellular spaces, together with detachment of epithelial basement membrane from underlying lamina propria. These histological changes appeared almost very weak in the capsule-treated group (Fig.1 f& g).

The number of intraepithelial lymphocytes in the lining epithelium of the villi of the IV group per 1000 µm^2 was about 24.0± 3.4 cells. This value was about 18.0± 3.6 cells per 1000 µm^{2}in the Capsule group. In addition, the number of lymphocytes in the connective tissue core was about 27.0±5.8 cells per 1000 µm^2 in the IV group. This value was about 19.0±3.6 cells per 1000 µm^2 in the capsule group. The number of lymphocytes infiltrated in the IV groups epithelial and connective tissue core is much higher than that of the Capsule group. The intestinal villi were interestingly oval to roundish in appearance.

Basophilic intraepithelial structures that are surrounded by a peripheral white halo. These structures measured about 15 µm in diameter and appeared at various levels among the epithelium, which were not observed in the villi of the (IV) group, which may be attributed to the local effect of 5- FU during its passage of the jejunum.

(Mashtoub, Feo et al.) found that intraperitoneal administration of 5-FU significantly decreased villus height and crypt depth in the Jejunum [3] and it was found that the mean villus height of the (IV) group measured about 260.0± 6.9 µm, which was significantly lower ($p<0.05$) than that of the Capsule group measured about 285.0± 9.3 in addition, the mean depth of crypts of IV group measured about 110.0± 6.8 µm which is significant ($p<0.05$) lower than that of Capsule group which measured about 141.0± 7.8 which means that, the degree of intestinal mucositis induced by the intravenous injection of 5-FU is significantly higher than that induced by that of orally administration of 5- FU microsponges formulation.

In addition, in the IV group, the number of goblet cells was about 16.0± 1.9 cells per villus compared to that of the capsule group, which was about 22.3± 2.4 cells per villus.(Ciobanu, Tantau et al) found that intraperitoneal injection of 5- FU at a

single dose of 500.0 mg/kg resulted in a severe reduction in the number of goblet cells in the group that received a lower dose of rifaximin compared to those that received a higher dose of rifaximin.

In the IV group, 5-FU injection resulted in a massive inflammatory response characterized by increased inflammatory cell infiltration in both mucosal and sub-mucosal regions. Additionally, massive deformities of villi, including shortening of height, loss, and atrophy, and marked disorganization of crypts and deformities of small intestine induced by 5-FU injection, these results were in agreement with those obtained by (Al-Asmari, Khan, et al.) who discussed the effect of ascorbic acid on the intestinal mucositis induced by the intraperitoneal injection of 5-FU however, There was no remarkable histological change observed in the animals of the (Capsule) group revealing that, the oral administration of 5-FU in the form of microsponges is safer than the intravenous administration of the drug.

Conclusion

The oral microspomges formulation of 5-FU is considered more safe inducing minimum degree of intestinal mucositis than that associated with the intravenous administration of commercially available 5-FU injection. Future studies should investigate the co-administration of the drug with either ascorbic acid or omega-3 fatty acids, as they have the ability to attenuate the intestinal mucositis associated with 5-FU[27,28].

Conflict of interests

There is no conflict of interest. The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

References

1. Benson III, A.B., et al., Recommended guidelines for treating chemotherapy-induced diarrhea. Journal of Clinical Oncology, 2004. 22(14): p. 2918-2926.

2. Labianca, R., et al., Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. The lancet, 1995. 345(8955): p. 939-944.

3. Mashtoub, S., et al., Oral nucleotides only minimally improve 5-fluorouracil-induced mucositis in rats. Nutrition and Cancer, 2015. 67(6): p. 994-1000.

4. Sonis, S.T., The pathobiology of mucositis. Nature Reviews Cancer, 2004. 4(4): p. 277.

5. Anderson, D.L., C. Chung-Heng, and S. Nacht, Flow characteristics of loosely compacted macroporous microsponge® polymeric systems. Powder technology, 1994. 78(1): p. 15-18.

6. Soares, P.M., et al., Inflammatory intestinal damage induced by 5-fluorouracil requires IL-4. Cytokine, 2013. 61(1): p. 46-49.

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

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

9. Joulia, J., et al., Determination of 5-fluorouracil and its main metabolites in plasma by high-performance liquid chromatography: application to a pharmacokinetic study. Journal of Chromatography B: Biomedical Sciences and Applications, 1997. 692(2): p. 427-435.

10. Bose, A., A. Elyagoby, and T. Wong, Oral 5-fluorouracil colon-specific delivery through in vivo pellet coating for colon cancer and aberrant crypt foci treatment. International Journal of Pharmaceutics, 2014. 468(1): p. 178-186.

11. Del Nozal, M., et al., Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications, 1994. 656(2): p. 397-405.

12. Matsushima, E., et al., Determination of S-1 (combined drug of tegafur, 5-chloro-2, 4-dihydroxypyridine and potassium oxonate and 5-fluorouracil in human plasma and urine using high-performance liquid chromatography and gas chromatography-negative ion chemical ionization mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications, 1997. 691(1): p. 95-104.

13. Anitha, A., et al., Combinatorial anticancer effects of curcumin and 5-fluorouracil loaded thiolated chitosan nanoparticles towards colon cancer treatment. Biochimica et Biophysica Acta (BBA)-General Subjects, 2014. 1840(9): p. 2730-2743.

14. Rustum, A.M. and N.E. Hoffman, Determination of 5-fluorouracil in plasma and whole blood by ion-pair high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications, 1988. 426: p. 121-128.

15. Petit, E., et al., Circadian rhythm-varying plasma concentration of 5-fluorouracil during a five-day continuous venous infusion at a constant rate in cancer patients. Cancer Research, 1988. 48(6): p. 1676-1679.

16. Harris, B., et al. Circadian periodicity of dihydropyrimidine dehydrogenase (DPD) activity in humans: possible rele vance to toxicity from fluoropyrimidine (FP) drugs with implications for FP infusion by programmable pumps. in Proc. Am. Soc. Clin. Oncol. 1988.

17. Tuchman, M., Sources of variability of dihydropyrimidine dehydrogenase activity in human blood mononuclear cells. Annual review of chronopharmacology, 1988. 5: p. 399-402.

18. Cohen, J., et al., Clinical pharmacology of oral and intravenous 5-fluorouracil (NSC-19893). Cancer chemotherapy reports, 1974. 58(5 Pt 1): p. 723.

19. Chirstophidis, N., et al., Fluorouracil therapy in patients with carcinoma of the large bowel: a pharmacokinetic comparison of various rates and routes of administration. Clinical pharmacokinetics, 1978. 3(4): p. 330-336.

20. Naguib, F.N., M.H. el Kouni, and S. Cha, Enzymes of uracil catabolism in normal and neoplastic human tissues. Cancer Research, 1985. 45(11 Part 1): p. 5405-5412.

21. Guo, S.R., et al., In vivo evaluation of 5-fluorouracil-containing self-expandable nitinol stent in rabbits: Efficiency in long-term local drug delivery. Journal of Pharmaceutical Sciences, 2010. 99(7): p. 3009-3018.

22. Tamaki, T., et al., Apoptosis in normal tissues induced by anti-cancer drugs. Journal of international medical research, 2003. 31(1): p. 6-16.

23. Vidon, N., et al., Hydroelectrolytic movements in rat jejunum during the alterations of the mucosa induced by a single injection of 5-fluorouracil. Digestion, 1979. 19(6): p. 370-374.

24. Ortiz, R., et al., 5-Fluorouracil-loaded poly (ε-caprolactone) nanoparticles combined with phage E gene therapy as a new strategy against colon cancer. International journal of nanomedicine, 2012.

25. Jain, V. and R. Singh, Design and characterization of colon-specific drug delivery system containing paracetamol microsponges. Archives of pharmacal research, 2011. 34(5): p. 733-740.

26. Ejam, A., et al., Histopathological assessment of antiulcerogenic effect of montelukast against acetylsalicylic acid induced gastric ulcer in male rabbit. Med. J. Babylon, 2015. 12(2).

27. Al-Asmari, A.K., et al., Ascorbic acid attenuates antineoplastic drug 5-fluorouracil induced gastrointestinal toxicity in rats by modulating the expression of inflammatory mediators. Toxicology reports, 2015. 2: p. 908-916.

28. Goto, T., et al., A comparative pharmacology study between the intracolonic and oral routes of 5-FU administration in a colon cancer-bearing Yoshida sarcoma rat model. Journal of pharmacological sciences, 2004. 95(2): p. 163-173.

Corresponding author**: Ahmed Usama Ali**

Department of Pharmaceutics, Faculty of Pharmacy, Merit University

Email: aasuoffi@hotmail.com

Phone: +20 1006579550