ABSTRACT

BACKGROUND

Ulcerative colitis (UC) is a chronic idiopathic, relapsing colon disease characterized by epithelial barrier disruption and inflammation of the colonic site. The inflammation involves the rectum to the anal margin and extends proximally into the colon; severe UC forms lead to colon cancer. The cause of UC remains unclear, although the interplay of Genetics, environmental and immunologic factors might be responsible. UC mainly affects people with an age group of 25–40 years of either sex. The occurrence of UC involved the activation of macrophage and dendritic cells. T-helper cells mediated the production of pro-inflammatory cytokines (IL-4, IL-5, IL-6, IL-12, IL-13 and TNF- α). Natural killer T-cells (NKT) have been associated with disrupting the epithelial cell barrier. Therefore the regulation of inflammatory cytokines leads to the recruitment of leucocytes which causes inflammation.

The treatment of UC is symptomatic. Mesalamine is a first-class of drug which is used in the treatment of UC with anti-inflammatory and antioxidant properties. It reduces pro-inflammatory cytokines and inhibition of COX-2 receptor and the antioxidant property of the drug reduces the Reactive Oxygen Species (ROS), ultimately reducing inflammation. Due to the rapid absorption of mesalamine from the small intestine, the systemic bioavailability of mesalamine shows many side effects like hepatotoxicity, cramping, headache, nausea, aching and vomiting due to the non-specific targeted delivery of mesalamine to the colonic region. The oral drug delivery system is one of the most preferred routes among the other routes. But due to the complex physiological system of the body as there is a difference in the pH of the G.I.T. compartments. The available conventional drug delivery systems cannot provide efficient drug delivery to the affected inflamed site. One of the major drawbacks of conventional drug delivery systems is non-targeted drug release**.** So there is an urgent need to develop a formulation with maximum efficacious and safer targeting drug systems, to attain the maximum concentration of a drug with minimum exposure to healthy tissue. As per the World Health Organization (WHO) definition, probiotics are ‘live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.’

The most commonly used commercial bacterial strains are *Lactobacillus*, *Saccharomyces* and *Bifidobacterium*. Firstly probiotics act as a safety barrier, and

they cover the intestinal tract near the brush border and do not allow the luminal bacteria from reaching the lamina propria and stimulating the mucosal immune system. Probiotics cause the mucosal immune system to secrete protective immunoglobulins (Ig) such as IgA and a host of protective bacteriocins and defensins into the lumen. The use of probiotics gives antioxidant and anti-inflammatory action. *S.boulardii* is a nonpathogenic strain of yeast. *S.boulardii* is the only yeast accepted as a human probiotic with positive effects in UC. As per the recent literature, it has been concluded that *S.boulardii* was as beneficial as mesalazine in reducing serum inflammatory factors and improving histological structure in dextran sodium sulfate-induced colitis in mice. To overcome the drawbacks of the conventional marketed formulations, formulation development aimed to design drug products for targeted drug release, reduction of frequency, and controlled release of drug in the inflamed colonic region. By considering the beneficial effects of mesalamine and the probiotic *S.boulardii,* we have planned to administer both in the same formulation as microparticles and pellets so that the probiotic may increase the therapeutic efficacy of mesalamine by giving an additive effect.

METHODS

Among many novel drug delivery systems attempted for colonic drug delivery, the microparticulate drug delivery system is one of the best approaches for controlled drug delivery in specific sites of inflammation. The ideal particle size for enhanced localization and increased drug residence time at the site of inflammation is between 4 and 15µm. In the present study, we have prepared micro-carrier formulations (microparticles and pellets) of Mesalamine and *S. boulardii* (probiotic) loaded in pectin (polysaccharides). The pectin microparticles were prepared by dehydration technique and coating was done using the oil-in-oil solvent evaporation method, whereas the extrusion formulated pellets–spheronization method and the coating of pellets was done by using the Accela-Cota technique. FTIR, DSC, SEM and XRD characterized both formulations. The *in vitro* release was studied in various simulated fluids, including SGF (pH 1.2, 900 ml), SIF (pH 6.8, 900 ml) and SCF (pH 7.4, 900 ml). The pharmacokinetic parameters were determined using High-Performance Liquid Chromatography (HPLC) in Wistar rat's plasma. The *in vivo*

pharmacodynamic studies were performed on Wistar rats using the TNBS Colitis model. The various parameters assessed were bodyweight evaluation, macroscopic characters assessment and diarrhea assessment. Also, hematology parameters and histopathological studies were performed to ensure recovery from the colitis disease. Finally, colonic enzymes MPO, LPO, and GSH concentrations were measured for the colitis severity estimation.

RESULTS

The optimized concentrations for microparticle formulation were in Mesalamine: pectin (1:3) ratio and 10^9 CFU of *S.boulardii*. The further coating was carried out using CAP as a coating polymer and acetone: ethanol (9:1) as the coating medium. The final ratio of 1:10 (CAP-coating medium) was selected as an optimized ratio for coating prepared microparticles. The mean particle size of the CAP-coated pectin microparticles was found to be 11.64±0.5 µm by using Zeta-sizer and the production yield was found to be 86.78%. The SEM evaluation ensured that the coated microparticles were spherical in shape.

Further, the XRD and FTIR evaluation confirmed that the drug and polymer have no significant interaction. *In vitro* dissolution studies revealed that almost 96% of the drug release was accomplished in 24 hrs in SCF at pH 7.4. In SCF presence during the 10^{th} hrs, uncoated and coated microparticles of *S.boulardii* showed $2.03x10^7$ and $2.07x10^7$ CFU/g viable count, respectively. The pharmacokinetic parameters were determined using HPLC and the observed mean C_{max} for the uncoated microparticles (12.13±0.52 mg/mL) was much higher than that obtained from the coated microparticles (5.91±0.69 mg/mL). Pharmacokinetic outcome found the controlled drug release manners of the optimized microparticles formulation, which recommends more capable management of colitis by supplying more drug concentration at the colonic site and decreasing the systemic drug absorption. In *in vivo* evaluation, it was found that the prepared coated microparticles significantly decreased lipid peroxides, myeloperoxidase and glutathione levels in colitis. In the Caco-2 cell culture model, the concentration of IL-8 is reduced considerably. The hematological observations confirmed that the prepared formulation showed a promising decrease in WBC, CRP and ESR levels in diseased animals. Animal experiments revealed that CAP-coated microparticles of mesalamine and *S.boulardii* significantly improved the colitis disease conditions of Wistar rats.

Pellets were formulated from blend powder consisting of 1.0% mesalamine and 10^9 CFU of *S.boulardii*, 29.0 % of pectin and 70.0% of MCC. Coating with 5% CAP w/v was optimized based on drug release and probiotics within 24 hrs. Physical mixtures of uncoated and coated pellets were evaluated for FTIR and DSC. It was found that there is no alteration in the spectra and DSC thermogram compared with the spectra and thermogram of pure drug. The peaks in XRD showed that uncoated Mesalamine pellets appear amorphous after coating with CAP. The peak intensity remains almost the same, indicating no interaction between the drug and polymers. Scanning Electron Microscopy (SEM) showed that uncoated pellets had a rough surface, but smooth spherical pellets were observed after CAP coating. The *in vitro* results confirmed that in CAP-coated pellets, only 13% drug release was observed within the first 5 hrs, indicating that the coating polymer can prevent premature drug release in the stomach and the small intestine.

During the 10^{th} hrs of dissolution in the SCF, uncoated and coated pellets of *S.boulardii* showed $2.01x10^7$ -$2.07x10^7$ CFU/g and $2.07x10^7$ CFU/g-$2.09x\ 10^7$ CFU/g viable counts respectively. The pharmacokinetic results confirmed that the C_{max} of uncoated pellets was 3.15±0.98 mg/mL and 1.06±0.37 mg/mL for coated pellets. This was comparatively more than indicating that less drug insertion. An *in vivo* evaluation confirmed that the prepared coated pellets containing mesalamine and *S.boulardii* could decrease the level of LPO and MPO in colitis and of GSH. The hematological observations confirmed that the prepared formulation showed promising decreased ESR levels in diseased animals. From the observed histopathological results, the CAP-coated microparticles and pellets of mesalamine and probiotic showed marked restoration of goblet cells, cytoplasmic mucin and crypt with slight inflammation. The overall appearance was similar to normal colon histopathology.

CONCLUSION

To manage UC, promising colonic drug delivery systems of Mesalamine and *S.boulardii* were developed. The developed pectin-based microparticles and pellets containing Mesalamine and *S.boulardii* were coated with CAP to minimize testing. The prepared drug delivery can also release the drug and probiotics in the specific colon-inflamed site. We demonstrated that the prepared coated microparticles and

pellets containing mesalamine and *S.boulardii* could decrease the level of LPO and MPO in colitis and increase GSH. The improvement in stool consistency and macroscopic scores ensured that combining mesalamine with *S.boulardii* significantly benefits the TNBS colitis model in Wistar rats. The *S.boulardii* shows additive effects when given with Mesalamine. The altered probiotic concentration in UC might be maintained by using the probiotic. Our study showed that the CAP-coated microparticles and pellets containing Mesalamine and *S.boulardii* have better therapeutic efficacy and reduce the side effects of the drug by loading it in a suitable drug carrier.

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