CHAPTER 5

"SUMMARY AND CONCLUSIONS"

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5.1 SUMMARY

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 in 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. 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 up 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 as well as inhibition of COX-2 receptor and antioxidant property of drug reduces the reactive oxygen species (ROS), ultimately leading to a reduction in 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 conferring 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 U.C. 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 conventional marketed formulations, formulation development aimed to design drug products for targeted drug release, reduction of frequency, and controlled drug release 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.

In the present study, we have prepared microparticulate formulations (microparticles and pellets) of mesalamine and *S. boulardii* (probiotic) loaded in pectin

(polysaccharides). The proposed delivery systems are novel approaches for preparing Mesalamine and *S. boulardii* (probiotic) loaded delivery systems for targeted Mesalamine and probiotic delivery at the colon for managing UC. Both delivery systems (pellets and microparticles) were evaluated for *in vitro* and *in vivo* activity estimation to ensure the efficacy of both prepared formulations.

Probiotic strain was selected based on antioxidant value and anti-inflammatory activity. It has been concluded that *S. boulardii* has better antioxidant and anti-inflammatory activity than *L. acidophilus*. Therefore S. *boulardii* was used for further studies.

In the present study, the pellets were formulated by the extrusion–spheronization method. The prepared pellets were coated with CAP by using accela-cota and characterized for drug loading, entrapment efficiency, micromeritics properties, pellets morphology by Motic microscope, pellets size, FTIR estimation, DSC, XRD , mucoadhesive, *in vitro* drug and probiotic release at pH 1.2, pH 6.8 and pH 7.4. Pharmacokinetic, pharmacodynamic, and *in vivo* studies, including assessment of weight, measurement of colonic enzymes estimation, estimation of ESR, C-reactive protein, WBC count, and histopathological evaluation.

The dehydration technique formed the microparticles. Oil-in-oil techniques coated the prepared microparticles. The prepared formulation was characterized for drug loading, entrapment efficiency, Shape and Surface Morphology, Particle size analysis, polydispersity index and zeta potential analysis, XRD, *in vitro* release of drug and probiotic at pH 1.2, pH 6.8 and pH 7.4. Pharmacokinetic, pharmacodynamic, and *in vivo* studies, including assessment of weight, measurement of colonic enzymes, estimation of ESR, C-reactive protein, WBC count and histopathological evaluation.

SUMMARY AND CONCLUSION

The summary of the whole study is given below:

Screening of the probiotic

- A Nitric oxide assay was performed to identify the antioxidant activity of *S. boulardii* and *L. acidophilus.* The results were conveyed as the dose necessary to cause 50% inhibitions by probiotic (IC_{50}).
- The IC_{50} value of *S. boulardii* and *L. acidophilus* was 58.06µg/ml and 71.11 µg/ml, respectively. The IC_{50} value between 50 µg/ml to 100 µg/ml is considered to ensure excellent intermediate antioxidant activity.
- The nitric oxide scavenging ability of *S. boulardii* was significantly higher than *L. acidophilus*. The result shows that *S. boulardii* exhibits admirable nitric oxide scavenging activity, which is satisfactory for further use in the formulation.
- For the anti-inflammatory effect evaluation of both probiotics (*S. boulardii* and *L. acidophilus*). The Caco-2 model of inflammatory cells was used.
- The inflamed Caco-2 cells treated with pro-inflammatory mediators contain significant amounts of IL-8. The concentration of IL-8 was decreased from 2591±167 pg/ml to 513± 76 pg/ml and 663± 49 pg/ml after stimulation, verifying the anti-inflammation properties of both probiotics.
- From the observed results, it has been concluded that *S. boulardii* has a better anti-inflammatory effect (513± 76 pg/ml) than *L. acidophilus* (663± 49 pg/ml).
- *S. boulardii* was used further in the formulation due to their better antioxidant and anti-inflammatory activity.

Preparation of microparticles and their evaluation

- Microparticles were successfully prepared by dehydration technique and coated by an oil-in-oil solvent evaporation method.
- The amount of pectin and surfactant varies and results were observed.
- The particle size analysis confirmed a relationship between the concentration of polymer and surfactant ratio.

- In the pectin microparticles, drug: pectin concentration (1:3) was found to optimally ensure the smaller microparticles with maximum drug loading and drug encapsulation.
- The core/coat ratio of 1:10 was selected as an optimized ratio for coating prepared microparticles with CAP.
- The particle size was 9.14±0.96 and 11.61±0.5 μm for uncoated and coated microparticles.
- The zeta potential of uncoated and coated microparticles was observed to be -26.78±4.66 *mV* and -29.36±3.36 *mV,* respectively.
- The polydispersity index of microparticles coated and uncoated was 0.245 and 0.267, respectively.
- Scanning electron microscopy (SEM) of prepared uncoated and coated micro-particles was performed. Uncoated microparticles were slightly spherical with a slightly porous character. An almost complete spherical shape without porous visibility with a smooth surface has been evaluated in CAP-coated microparticles.
- The XRD peak showed that the intensity remains almost the same in CAP-coated microparticles, which confirmed no chemical reaction in drug and polymers.
- The compatibility between drugs and excipients was assessed through infrared spectroscopy.
- These findings revealed that all the major peaks corresponding to Mesalamine were in IR spectra of uncoated and coated microparticles. It indicated that Mesalamine is fully compatible with polymers without any significant interference.
- This dissolution study was performed to mimic the gastrointestinal tract conditions as our main aim was to prepare colon-targeted drug delivery systems of Mesalamine and *S. boulardii* .
- The prepared uncoated and coated microparticles were placed in different

simulated gastrointestinal fluids. In the case of a plain drug, immediate Mesalamine release was observed in the initial 1 hrs. More than 65% of the Mesalamine was released in the first 2 hrs in 0.1 N HCl (SGF) and around 90% of the Mesalamine was released within 6 hrs (SIF).

- In the case of uncoated microparticles, around 40% of the mesalamine release was observed in the first 2 hrs in 0.1 N HCl (SGF).
- More than 70% of mesalamine release was observed within 6 hrs (SIF). Around 90% of the mesalamine release was observed within 10 hrs in SCF. In the case of coated microparticles, only 2-3% of the mesalamine release was observed in the first 2 hrs in 0.1 N HCl (SGF).
- Due to the enteric coating of microparticles, CAP can restrict mesalamine released in the stomach. This small quantity of drug release through this period may be due to the presence of the un-entrapped drug on the surface of the microparticles or diffusion of the drug from the outer surface of the pectin microparticles.
- Around 7% of the mesalamine release was observed within the first 4 hrs. More than 35% of the mesalamine release was observed within 6 hrs due to the removal of coating of CAP in SCF. About 70% of the Mesalamine release was observed within 10 hrs. Almost 96% of the mesalamine release was accomplished within 24 hrs in SCF at pH 7.4.
- The uncoated microparticles showed that about 50% of probiotics were released in 2 hrs in SGF at pH 1.2. Almost 75% of probiotic was released in SIF (pH 6.8) in 5 hrs of uncoated microparticles. Approximately 90% probiotic release was observed in 10 hrs at a pH of 7.4 SCF.
- Near about 70% of the probiotic release was observed within 8 hrs. In the case of coated microparticle formulation in the first 2 hrs, in SGF at pH 1.2, only 2-4 % probiotic release was observed. At the 4^{th} hrs pH 6.8, about 25% of probiotic releases were observed due to the eruption of coating polymer by SIF.

- Approximately 96% of probiotic release was observed within 24 hrs. A combination of mesalamine observed a slight rise in viable cell count and *S. boulardii* in pectin-based CAP-coated microparticles confirming that this combination can be used further *in vivo* studies.
- In SCF presence during the 10^{th} hrs, uncoated and coated microparticles of *S. boulardii* showed $2.03x10^7$ CFU/g and $2.07x10^7$ CFU/g viable count, respectively.

Preparation of pellets formulation and evaluation

- Mesalamine and probiotic-loaded pectin pellets were prepared by the extrusion–spheronization method.
- Pellets were formulated from 5 grams of blend powder consisting of 1.0% Mesalamine, 10^9 CFU of *S. boulardii*, 29.0 % of pectin, and 70.0% of MCC.
- The choice of the most excellent formulation was based on pellet size, % age yield, angle of repose, entrapment efficiency and friability. After obtaining the desired results Moisture content: 4.33±0.04, Percentage yield: 86.33±0.19, Average particle size: 518±20.2µm the batch MP10 was selected for further coating process and tagged as MP11.
- Coating of prepared pellets was done by using an accela-cota instrument.
- 5% of the CAP concentration of coating material provides a smooth surface with desired *in vitro* Mesalamine and probiotic release.
- The optimized pellets sizes of batches MP8, MP9 and MP10 and MP 11 were observed to be 541µm, 463µm, 518µm and 513µm correspondingly. Size and shape analysis was done for different pellets formulations.
- Flow properties results including Angle of repose (24.213±0.361), Bulk density (0.776±0.005), Tapped density (0.840±0.020), Hausner's ratio (1.084±0.03) and Carr's index (8.516±0.562) of different formulations of pellets are within the acceptable range.

- The friability test was conceded for optimized pellets formulations to ensure their mechanical strength. The uncoated and coated formulations showed good mechanical strength (0.49±0.381).
- Drug-loaded pellets entrapment efficiency was determined and repeated thrice. The percentage entrapment efficiency was observed to be 85.31%±0.11 and 82.67%±0.05 for uncoated pellets and coated pellets, respectively.
- It was evident from the results that all the major peaks were present in FTIR spectra of uncoated as well as coated pectin-based mesalamine and probiotic-loaded pellets. These findings indicate no primary interface between the drug and used excipients. There was no sign of any degradation.
- From the DSC results, it has been observed that the peaks ensured no major interaction between drug and polymers.
- The peaks in XRD showed that uncoated Mesalamine pellets appear amorphous after coating with CAP. The peaks' intensity remains almost the same, indicating no chemical interaction between the drug and polymers.
- Scanning electron microscopy (SEM) of prepared uncoated and coated pellets was performed. Uncoated pellets were slightly spherical in shape but a smooth spherical pellet has been observed after CAP coating.
- The quantitative attachment of pellets on excised intestinal tissue has been evaluated. A huge pellets number on the mucosa specified an enhanced adhesion of the pectin-based pellets.
- Approximately 100% of pellets got adhered to the mucosa. The maximum adhesion ability of uncoated pellets on rat mucosa showed that the pectin pellets formulation has good mucoadhesion properties.
- For an efficient colonic drug delivery system, it has to reside unchanged in the upper G.I.T. For plain Mesalamine dissolution, immediate drug release was observed.
- More than 60% of the Mesalamine was released in the first 2 hrs in simulated

gastric fluid and around 80% of the Mesalamine was released within 5 hrs in simulated intestinal fluid.

- In uncoated pellets near about 40% of the Mesalamine release was observed within the first 2 hrs in SGF. In contrast, uncoated pellets fail to meet the criteria in preventing premature Mesalamine release in SGF and about 62% of the Mesalamine was released within 5 hrs and more than 90% of the Mesalamine release was observed in 10 hrs. In CAP-coated pellets (MP11), only 4% of the Mesalamine was released in the first 2 hrs in SGF.
- Due to the enteric coating of pellets, CAP can restrict Mesalamine released in the stomach and about 13% of the Mesalamine release was observed within 5 hrs in SIF. After 10 hrs, more than 55% of the Mesalamine has been released and almost the total amount of mesalamine release was accomplished in 24 hrs in SCF at pH 7.4. In the case of uncoated pellets, the *in vitro* Mesalamine release results showed that around 65% of the Mesalamine released was observed within the first 5 hrs.
- But in CAP-coated pellets, only 13% of the mesalamine release was observed within the first 5hrs, indicating that the coating polymer can prevent premature Mesalamine released in the stomach and the small intestine. A significant difference between the release pattern was observed from coated and uncoated pellets containing mesalamine and probiotics.
- For uncoated formulation, almost 25% of the probiotic was released in 2 hrs in SGF at pH 1.2. More than 70% of the probiotic was released in SIF (pH 6.8) in 5 hrs. Approximately 80 % of the probiotic release was observed in 8 hrs at pH of 7.4 SCF.
- In the case of coated pellets formulation in the first 2 hrs, in SGF at pH 1.2, only 2-4 % of the probiotic release was observed. At the 4^{th} hrs pH 6.8, around 20% of the probiotic releases were observed due to the eruption of coating polymer by SIF.

- But after 6 hrs immediate release effect in the probiotic release was observed. The results were expressed as log CFU/g of yeast. A combination of mesalamine noticed a minor rise in viable cell count and *S. boulardii* in CAP coated pellets revealing that this formulation might be utilized further for *in vivo* studies.
- During the 10^{th} hrs in SCF presence, uncoated and coated pellets of *S. boulardii* Showed $2.01x10^7$ CFU/g -$2.07x10^7$ CFU/g and, $2.07x10^7$ CFU/g -$2.09x\ 10^7$ CFU/g viable counts respectively.
- A slight rise in viable probiotic cell count was observed in coated pellet release, confirming that the coated pellets can release the probiotic to the specific colonic region.

5.2 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 so premature drug and probiotic release in the stomach and small intestine can be minimized. The prepared drug delivery systems 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 the combination of 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.