In vivo evaluation of curcumin loaded granules using Eudragit FS30D and Guar-gum coating in the treatment of ulcerative colitis in albino rats

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Ulcerative colitis (UC) is a wide-reaching health problem with unknown etiology and its high mortality rates. Conventional therapy for onset treatment of active UC includes immunomodulators, corticosteroids, purine analogues, antibiotics and 5-aminosalicylic acid. Unfortunately, after giving long term treatment to the patients associated with UC, these drugs are found to have undue side effects. There is a need for novel therapeutical agents which is least toxic, effective and economical. Experimental data from the preclinical studies suggested that curcumin, a natural polyphenol molecule derived from the Curcuma longa is effective in preventing ulcerative colitis. The present work is divided into two sections: 1) multiparticulate delivery of curcumin loaded granules to the colon and it’s in vivo evaluation, 2) co-administration of curcumin along with probiotics to demonstrate significant protective action against acetic acid-induced UC in rats. Experimental research work is mainly focused on the induction of colitis, assessment of colitis, diarrhoea assessment, change in body weight, fecal bleeding assessment, blood analysis and histopathological study of colonic sections. In conclusion, our results suggest that the co-administration of probiotics along with colon targeted delivery of curcumin showed potential beneficial protective effect against acetic acid induced UC in rats.

Keywords: Curcumin, Ulcerative colitis, Guar-gum, Polysaccharides, Probiotics
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Ulcerative colitis (UC) is an idiopathic inflammatory bowel disorders with unknown etiologic having characteristic features of inflammation of mucosal and sub mucosal part of the colon and rectum of the gastrointestinal tract. This disease is full of complications having diverse features and that's why it is also called “complications of the disease”. Although the pathogenesis of UC remains unclear but combination of an environmental, genetical and immunological factors are partially responsible for the ulcerative conditions. Imbalances among pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), Interleukin (IL)-1, IL-6, and IL-12, and anti-inflammatory cytokines, such as IL-4, IL-10, IL-11, are believed to play very crucial role in the modulation of inflammation. There can be seen imbalance of intestinal beneficially acting bacteria triggered an inappropriate, overactive, and ongoing mucosal immune response which lead to the intestinal tissue damage in an individual. Blood loss and mucus mixed with stool from the inflamed intestines accompanied by lower abdominal cramping during the passage of bowel movements is the most common symptoms of ulcerative colitis. From the decades, drugs like adrenocorticosteroids, immunomodulators, corticosteroids, purine analogues, antibiotics and 5-aminosalicylic acid are the mainstays of medical therapy. These medicaments are available in a variety of forms are used an orally and topically to reduce inflammation of the colon and rectum. Nonetheless, all these drugs are useful for treatment tolerated with number of short comings side-effects. However, treatments with these medications is typically accompanied with adverse side effects such as nausea, dizziness, changes in blood chemistry (including anaemia and leukopenia), skin rashes and drug dependence. There are numerous side-effects associated with these modern system of medication and hence there is a need to move to an alternative therapy that may have more or equally effective besides being at cost-effective price. To reduce these side effects and increase the pharmacological efficacy, plants or Ayurvedic based formulation can

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be used as an alternative therapy\textsuperscript{10}. Extensive research from the last decades has revealed the applications of curcumin as an inflammatory diseases in prevention of UC\textsuperscript{11,12}.

Curcumin, a natural polyphenol used in the dietary spices of most of the Indian kitchen, extracted from the dried rhizomes of the perennial herb turmeric \textit{Curcuma longa} L. (Family: Zingiberaceae). Curcumin is used as a spice to give the specific flavour and yellow colour to curry\textsuperscript{13}. Study reports from the different researcher showed that curcumin exerted preventive and therapeutic effects in models of colitis by inhibiting NF-κB activation\textsuperscript{14-17}. Literature evidences demonstrated that number of researchers formulated colon targeted drug delivery by using xanthan gum and guar-gum to overcome the side-effects caused by drugs in the upper gastro intestinal tract\textsuperscript{18-20}. An aspect that has been largely ignored is that of the cytotoxic effects of these drugs that may exert on the colonic bacteria that are involved in the metabolism of these gums (xanthan gum and guar gum) and the release of the drugs in the subsequent doses. Therefore, the subsequent doses may not be able to release the drug completely\textsuperscript{21,22}. By understanding the disturbed microbial ecology of GIT by oral administration of drugs, our aim of the study was to design and deliver the combinational therapy of treatment as an alternative medicament. It will help in mimicking the function of beneficial GIT bacteria. The main focus of the present study was to develop novel formulations for site specific delivery of curcumin to the colon using guar gum and xanthan gum as a carrier. The problem of the compromise in the colonic bacteria will be taken care of by administering probiotics. These probiotics will not only restore the bacterial picture to normal but will also help in metabolism of the coating gums leading to release of the drug in the colon to exert its carcinogenic action.

### Methods and materials

All the chemicals and reagents used in the present study were of analytical grade. The active material (Curcumin) was purchased from K Patel Phyto Extractions Pvt. Ltd, Mumbai, India. \textit{Guar-gum} having a viscosity of 5200-5500 cps and Xanthan gum were obtained from Molychem Manufacturers (P) Ltd, Mumbai. Eudragit FS 30D was an Evonik Industries (Germany) product. All the glassware were washed in dilute nitric acid and thoroughly washed with double distilled water and dried in hot air oven.

### Preparation of curcumin loaded formulation

The granules of curcumin containing equal composition of drug to polymer ratio were prepared by a dry granulation method. The composition of curcumin, \textit{guar-gum} and xanthan gum were at a ratio of 1:1:1. Polymers were passed separately through sieves No. 80 and were mixed with curcumin. The prepared granules were coated up to 20% with a \textit{guar-gum} dispersion of 4% w/w in ethanol/water in an accelacota coating pan to a definite cumulative coating weight gain. After coating, the granules were dried for 5 hrs at 65 °C. The inner coating with \textit{guar-gum} offers an additional protection to the multi particulate unit, until it is degraded by microbial enzymes at the proximal colon. Further, the \textit{guar-gum} coated granules were again coated with 40% of Eudragit FS 30D in an accelacota coating pan. Outer coating with Eudragit FS30D, works as a time-controlled retardant and offers additional protection to the drug released from its dosage form.

### In vivo evaluation of curcumin loaded formulation

#### Experimental animals

Thirty adult Wistar albino rats (either sex) with weight range 246-255 gm were obtained from the Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology. The animals were allowed to acclimatize under standard environmental conditions. The animals were housed in the animal house in groups of five animals each in clean poly-acrylic cages and maintained 12 hrs day and light cycles at an average ambient temperature of 25± 2 °C and 60±10% relative humidity. Animals had free access to water and standard pellet diet during the exposure of treatment, except short fasting period of 4 hrs after the intracolonic administration of 2 % v/v acetic acid. The study protocol was approved by the Institutional Animal Ethics Committee of Birla Institute of Technology vide Approval Number BIT/PH/IAEC/06/2014.

#### Induction of colitis procedure

UC was induced by the use of dilute acetic acid\textsuperscript{23,24}. Rats were anesthetized with a dose of 75 mg/kg ketamine injection (i.p.). A flexible plastic catheter with an outer diameter of 2 mm was then inserted rectally into the colon with the aim to place the catheter tip 8 cm proximal to the anus. Colitis was induced by intra-colonic instillation of 2 ml/day 2 % v/v acetic acid with 24 hrs interval for three constitutive days. Injected rats were maintained in a
head-down position for 2 min to prevent solution leakage. The rats were inspected individually for the presence of diarrhoea and bloody stool. On day 0th, animals were randomly divided into 6 groups each of 5 animals. After induction of UC in rats, they were subjected to 15 days of dosing period. The dosages were freshly prepared every day by suspending the drug/formulation in milk and administered orally once daily. The distribution of groups and dosage schedule are given in Table 1.

Assessment of colitis

The various formulations mentioned in (Table 1) were evaluated for their anti-ulcerogenic effect on acetic acid induced UC in rats. The anti-inflammatory response was assessed by measuring various clinical parameters like change in body weights, change in organ weights, presence/absence of bloody diarrhoea and histopathological examination of colonic tissue.

Diarrhoea assessment

After instillation of 2 ml of 2% w/v acetic acid for induction of colitis in rats on each day, they were housed singly in cages with clean white sheet at the bottom of the cage and observed for 4 hrs for presence of onset of diarrhoea, number of fecal matter discharge, consistency of fecal content, fecal bleeding, diarrhoea assessment (ranking of diarrhoea according to the faeces consistency). Also during the drug treatment fecal matter from each group were examined daily in the morning for the assessment of diarrhoea. The recordings were done and ranked according to grading given by Gibson et al., (2003 & 2005) in Table 2.

Change in body weight

The body weights of animals were recorded initially before induction of UC (0th day), after induction of UC (after 5th day), 10th day, and final day (15th day) before sacrificing the animals.

Fecal bleeding assessment

The presence of blood in the faecal matter was assessed by performing strip test. The faecal content of rats was collected daily during induction of colitis and during the treatment period, then it was dispersed in water and blood content was qualitatively measured with analysis strips taking the change in colour of the strip as the parameter for blood indicator.

Histological evaluation

Animals were sacrificed at the end of the 15th day treatment and colonic tissue samples were taken and fixed in freshly prepared 10% formalin solution. The samples were examined histopathologically to study the anti-ulcerogenic effect of various formulations used for the treatment of UC as mentioned in Table 1. The transverse sections of colonic tissues were taken and embedded in paraffin blocks. These sections were stained with haematoxylin and eosin. The slides were examined microscopically for patho-morphological changes such as congestion, haemorrhage, inflammation, erosion and ulceration in the mucosal, sub mucosal, muscular and serosal layers of colon specimen.

Statistical analysis

Statistical comparison was performed using either unpaired t-test and for multiple comparisons versus control group followed by Dunnett's test. All statistical analysis was performed using SPSS. *P*<0.05 was considered statistically significant.

Results and discussion

Effect on animal weight

Table 3 shows the mean body weight of UC induced animals exposed to different treatments. The body weight of animals exposed to the treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (p.o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>2 %v/v Acetic acid</td>
<td>2 ml/kg (intracolonic)</td>
</tr>
<tr>
<td>Group II</td>
<td>Meslamine powder + 2 %v/v Acetic acid</td>
<td>23 mg/kg + 2 ml/kg</td>
</tr>
<tr>
<td>Group III</td>
<td>Curcumin+ 2 %v/v Acetic acid</td>
<td>500mg/kg +2ml/kg</td>
</tr>
<tr>
<td>Group IV</td>
<td>Curcumin formulation + 2 %v/v Acetic acid</td>
<td>500mg/kg + 2ml/kg</td>
</tr>
<tr>
<td>Group V</td>
<td>Curcumin formulation + Probiotics + 2 %v/v Acetic acid</td>
<td>500mg/kg +1 gm/kg +2ml /kg</td>
</tr>
<tr>
<td>Group VI</td>
<td>Normal control</td>
<td>50mg/kg (water)</td>
</tr>
</tbody>
</table>

Table 2—Ranking of diarrhoea

<table>
<thead>
<tr>
<th>Rank</th>
<th>Type of diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No diarrhoea</td>
</tr>
<tr>
<td>1</td>
<td>Mild diarrhoea (staining to anus)</td>
</tr>
<tr>
<td>2</td>
<td>Moderate diarrhoea (staining over top of the legs and lower abdomen)</td>
</tr>
<tr>
<td>3</td>
<td>Severe diarrhoea (staining over legs and higher abdomen, often with continual anal leakage)</td>
</tr>
</tbody>
</table>
scheduled of acetic acid induced UC was found to be decreased in comparison to normal. The percentage change in body weight of the group I animals decreased (246.6±7.18 and 230.4±7.10) as compared with 0th and 15th day of treatment with respect to study design. Body weight results of other groups, i.e., II, III, IV and V showed marked degradation in body weight at 15th day of treatment when compared with day of induction of ulcerative colitis. Animals of group V and VI showed comparable increment in body weight at 15th day of treatment as compared to their initial weights. There is an increase in the body weight of animals of group V which have been exposed to the formulation of curcumin along with probiotics in respect with group VI. Body weight gain during drug treatment for any kind of disease is generally considered as the reflection of improvement of the disease.

**Faecal bleeding assessment**

The presence of blood in the faecal matter of animals subjected to 2% v/v acetic acid treatment, was assessed by performing strip test. Faecal content was dispersed in water and strip was dipped into it for 60 seconds and change in the colour of the strip is noted, which gives the number of RBC, i.e., ca cells/µl of the cecal content, which can be categorized into small, moderate and large amount of RBC concentration.

**Blood analyses**

The effect of 2% v/v acetic acid induced UC on the RBC is shown in Table 4. The study carried out on various treatments reveal changes in RBC count after 15th day oral treatment. All treatment group animals showed reversal of decrease in red blood cell count. Group-V animals showed maximum increase (8.65%) in red blood cell count when compared with post UC values. However, the probiotics treatment group animals showed RBC count comparable to UC control group animals. Restoration of RBC in the blood is an important sign of cure or ulcers.

**Table 3—Body weight of animals**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Treatment given</th>
<th>0th day</th>
<th>5th day</th>
<th>10th day</th>
<th>15th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group 1</td>
<td>2 % v/v Acetic acid</td>
<td>246.6±7.18</td>
<td>239.0±5.09</td>
<td>236.6±5.60</td>
<td>230.4±7.10</td>
</tr>
<tr>
<td>2</td>
<td>Group 2</td>
<td>MES-powder +2 % v/v Acetic acid</td>
<td>249.5±9.18</td>
<td>249.5±5.76</td>
<td>247.0±5.46</td>
<td>242.6±10.01</td>
</tr>
<tr>
<td>3</td>
<td>Group 3</td>
<td>Curcumin + 2 % v/v Acetic acid</td>
<td>250.5±11.2</td>
<td>249.0±5.78</td>
<td>247.1±5.33</td>
<td>245.5±11.10</td>
</tr>
<tr>
<td>4</td>
<td>Group 4</td>
<td>Curcumin formulation + 2 % v/v Acetic acid</td>
<td>252.4±12.2</td>
<td>252.2±4.80</td>
<td>251.4±7.75</td>
<td>249.6±4.97</td>
</tr>
<tr>
<td>5</td>
<td>Group 5</td>
<td>Curcumin formulation + Probiotics + 2 % v/v Acetic acid</td>
<td>252.4±13.4</td>
<td>256.8±4.95</td>
<td>258.2±6.22</td>
<td>259.0±7.51</td>
</tr>
<tr>
<td>6</td>
<td>Group 6</td>
<td>Normal control</td>
<td>252.0±12.3</td>
<td>253±5.41</td>
<td>258.5±5.25</td>
<td>259.8±7.80</td>
</tr>
</tbody>
</table>

**Table 4—Blood parameters of different group before induction of UC at 0th and 15th day**

<table>
<thead>
<tr>
<th>Blood parameters (Day)</th>
<th>2 % v/v Acetic acid</th>
<th>MES-powder +2 % v/v Acetic acid</th>
<th>Curcumin + 2 % v/v Acetic acid</th>
<th>Curcumin formulation + 2 % v/v Acetic acid</th>
<th>Curcumin formulation+ Probiotics + 2 % v/v Acetic acid</th>
<th>Normal control</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (0)</td>
<td>7.45</td>
<td>7.66</td>
<td>8.32</td>
<td>7.38</td>
<td>8.65</td>
<td>7.74</td>
</tr>
<tr>
<td>RBC (15)</td>
<td>6.21</td>
<td>7.23</td>
<td>6.72</td>
<td>6.10</td>
<td>6.54</td>
<td>6.92</td>
</tr>
<tr>
<td>WBC (0)</td>
<td>6.22</td>
<td>5.67</td>
<td>6.72</td>
<td>8.89</td>
<td>8.90</td>
<td>6.40</td>
</tr>
<tr>
<td>WBC (15)</td>
<td>11.02</td>
<td>10.20</td>
<td>8.32</td>
<td>8.89</td>
<td>8.30</td>
<td>8.30</td>
</tr>
</tbody>
</table>
administration. Further treatment with various formulations indicated the reverse of WBC count.

**Histopathology of colon sections (Fig. 1)**

**Group I:** In the histopathology studies, the colon tissue of the animal of group I, a complete destruction of mucosa and cryptic architecture was observed. Presence of inflammatory cells in mucosal layers and RBC congestion along with surface erosion indicates the presence of acute disease. The animals of only this group were found to have lost weight at the end of the study. In rest of the groups although a decrease in mean body weight of animals was observed after the induction of colitis. Recoveries was seen with time and at the end of the study the average body weight of all the groups were found to be higher than those at the zero day. On similar lines, though a decrease in RBC levels and an increase in WBC levels could be observed in all the groups after the induction of disease, a recovery of the cell counts could be observed in animals of all the groups.

**Group II:** The colonic specimen of group II showed mild deformation of mucosal layer and disruption of epithelium at some places. Inflammation with moderate infiltration of inflammatory cells was also observed. The regeneration of crypts was observed at certain places. The picture shows that the partial regeneration of the damaged tissue was there indicating the therapeutic effect to the drug.

**Group III:** The colonic specimen of group III treated with powder of curcumin showed less damaged of colonic mucosal layer as compared to animals of group I and II. The goblet cells appear decreased as compared to normal control. The mucosal layer shows scattered infiltration by mononuclear inflammatory cells predominantly comprising of lymphocytes and histiocytes. The submucosal layer, muscular and serosal layers appear within normal limits. The partial recovery is also indicated by the reversal of weight loss and cell count.

**Group IV:** The colonic section of group IV studied shows that intact intestinal mucoa. Intervening the glands in the mucosal layer, there are seen mild infiltration by inflammatory cells predominantly comprising of lymphocytes, plasma cells and macrophages. The submucosal layer shows moderate edema. The muscular and serosal layers appear unremarkable.

**Group V&VI:** Interestingly, the histopathological results of the colon tissues taken from the animals of both group V&VI were similar showed near to normal condition of both the specimens. The much better healing in the case of group V&VI as compared to animals of other group II&III clearly demonstrate the regenerative effect of both prebiotics (Guar-gum and Xanthan gum). These histopathological findings from the colonic specimen of both the group showed the
intact colonic mucosa with intact lining epithelium. The mucosal layer is focally infiltrated by mononuclear inflammatory cells predominantly comprising of lymphocytes and histiocytes. The submucosal layer, muscular and serosal layers appear within normal limits.

Recent studies on *Pulsatilla chinenis* (Bunge) Regel. have also been done where colon-targeted particles coated with Eudragit S100 were administered and examined for ulcerative colitis and protective effect of alcoholic extract of stem of *Entada pursaetha* in dextran sulphate sodium-induced colitis in mice were also examined.\(^{27,28}\)

**Conclusion**

Curcumin is used as an anti-inflammatory agents. A number of literature evidences are available, where the colon targeted delivery of curcumin caused serious systemic side effects like mucositis, diarrhoea, alteration of normal microflora, and translocation of bacteria has been claimed. So, certain manipulation of human microflora is needed in the colon for maintaining the number of beneficial bacteria. This problem of alteration of normal microflora was overcome by concomitant administration of targeted formulation along with probiotics. Consistency of caecal was much less in rats treated with free powder of mesalamine, curcumin and 2% v/v of acetic acid. But caecum contents was found to be normal in case of others. From histopathological study, diffuse ulceration and focal ulceration were observed in mesalamine, curcumin and 2% v/v of acetic acid treated rats. Rest of the groups in which concomitant administration of probiotics was done showed intact colonic mucosa with intact lining epithelium. The study also offers a solution to the problem in term of concomitant administration of probiotics along with colon targeted drug delivery of curcumin. This approach offers the advantage of minimizing the other GIT related side-effects like diarrhoea, ulceration and mucositis.

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**References**


