Available online on 15.03.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research



Open Access Research Article

# Synthesis, in-vitro characterization and pharmacological evaluation of conjugates of flurbiprofen and polysaccharides for colon specific drug delivery

**P. Soni, L. K. Soni, G. P. Choudhary ***

School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (MP), India

## ABSTRACT

The aim of the study was to prepare site specific drug delivery of flurbiprofen using polysaccharides by the formation of glycosidic linkage which is hydrolysed by the microflora present in colon. This approach prevents drug release in the upper gastrointestinal environment. Due to the minimal degradation of conjugates in upper Git, the in vitro drug release in SGF, SIF and SCF was found upto 4.26±0.03%, 12.41±0.08% and 92.72±3.33% respectively.

**Keywords**: Colon specific drug delivery, Conjugates, Flurbiprofen, Microbial system.

**Article Info:** Received 04 Feb 2019; Review Completed 09 March 2019; Accepted 12 March 2019; Available online 15 March 2019

**Cite this article as:**

Soni P, Soni LK, Choudhary GP, Synthesis, in-vitro characterization and pharmacological evaluation of conjugates of flurbiprofen and polysaccharides for colon specific drug delivery, Journal of Drug Delivery and Therapeutics. 2019; 9(2):316-320 **http://dx.doi.org/10.22270/jddt.v9i2.2425**

***Address for Correspondence:**

Dr. G. P. Choudhary, Associate Professor, School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (MP), India

## INTRODUCTION

Colon specific drug delivery technologies (CSDDT) are being enormously improved in current years. The major impediments in drug targeting to the colon are degradation pathways and systemic absorption of the drugs in the upper GI tract. Many research studies are being done in CSDDT to meet the needs of ever-increasing gastrointestinal complications such as Crohn's disease and ulcerative colitis[1-3] amebic colitis [4-5], to treat colon cancer [6-9], to deliver the protein and peptide drugs[10] and for the healing of ailments such as nocturnal asthma [11, 12], early morning arthritis [13,14], nocturnal angina [15] and hypertension [16] influenced by circadian rhythms. A range of strategies/systems have been developed/practiced for better colonic drug delivery. Among all other strategies, The traditional approaches for colon targeting are prodrug formulation, pH-sensitive drug delivery, time-dependent systems and microbial degradation methods to formulate different dosage forms like tablets, capsules, multiparticulates, microspheres, liposomes. . microbial degradation system is more frequent and acceptable because A number of synthetic azo polymers and natural or modified polysaccharides (chondroitin sulphate, guar gum, xanthan gum, locust gum, inulin, dextrans, starch, amylose,pectins) degraded by the human colonic flora, have thus been investigated as colonic drug delivery carriers. The human colon has over 400 distinct species of bacteria as resident flora, a possible population of upto millionsof bacteria per gram of colonic contents. Among the reactions carried out by these gut flora are azoreduction and enzymatic cleavage i.e. glycosides. These metabolic processes may be responsible for the metabolism of many anti-inflammatory drugs and may also be applied to colon targeted delivery of them. .These systems are designed to reduce/avoid the degradation and absorption of the drugs in the starting portion of the gastrointestinal tract and reduce side effects of drugs. In oral colon-specific drug delivery system, colon has a large amount of lymphoma tissue (facilitates direct absorption in to the blood), negligible brush boarder membrane activity, and much less pancreatic enzymatic activity as compared with the small intestine to treat local diseases associated with colon. Nonsteroidal anti inflammatory drugs (NSAIDs) are extensively used in the management of pain and inflammation associated with osteoarthritis, rheumatoid arthritis and ulcerative colitis. They also preferred for the management of inflammatory bowel disease and large intestine cancers [17,18]. Flurbiprofen, a nonsteroidal anti-inflammatory drug, successfully used for the management of inflammation, pain associated with rheumatoid arthritis, was selected as a model drug. It has plasma half-life 3-6 h. Its high dose frequency is due to its shorter half-life [19]. The polysaccharides as carrier of NSAIDs to target colon is much better approach by formation of

glycosidic conjugates. It is biocompatible, biodegradable, non-toxic and shows mucoadhesive properties as well. Due to its mucoadhesion nature, its residence time in the colon can be increased, which subsequently results in maximum bioavailability. Taking the above information into account, a study was designed for the preparation and characterization of flurbiprofen conjugates for the colon drug delivery system[20].

## METHODS AND MATERIALS

### Materials

Flurbiprofen selected for the project was procured as gift samples from FDC, Ltd, Mumbai ,Indi Polysaccarides like dextran was purchasd from Himdia, Mumbai, India; chitosan was obtained as gift sample from Centeral Institute of Fisherish Technology, Cochin, India. Pectin and Inulin was purchased from Loba Chemical Pvt. Ltd, Mumbai. All other chemical and reagent were used of analytical grade.

### Methods

### Preparation of Drug Conjugates[21-23]

### Chemical modification of flurbiprofen

### Reduction of flurbiprofen

Flurbiprofen ( 0.03 mole) and LiAlH4 ( 0.03 mole) in diethyl ether were refluxed in a water bath for 2 hr, filtered, washed with 10 mL diethyl ether for three times and then finally washed with water, dried and purified by recrystallization using ethanol water. Yield 71%; M.P. 107-109 $^{o}$C.

### Chlorination of the [2-(2-Fluorobiphenyl-4-yl)] Propenol:

The compound [2-(2-Fluorobiphenyl-4-yl)propanol] ( 0.015 mole) and thionyl chloride (1.6 mL, 0.015 mole) were mixed and allowed to get reacted in a round bottom flask at 0 $^{o}$C for 1 hr, refluxed for 2 hr and allowed to cool and poured on to ice cold water. The compound was washed with distilled water several times to remove any unreacted thionyl chloride, purified by recrystallization using ethanol-water and dried. Yield 62%; M.P. 120- 122 $^{o}$C.

### Conjugation of modified naproxen with polysaccharides

### Synthesis of Dextran-Flurbiprofen Conjugate (DFl)

The solution of dextran in 25 mL of DMF in a round bottom flask, [2-(2-Fluorobiphenyl-4-yl) 1- Chloro propane ( 0.008 mole), mercuric bromide ( 0.008 mole) and yellow mercuric oxide ( 0.004 mole) were added and refluxed for 2 hr under stirring. The obtained suspension was filtered and the filtrate was dried in a rotatory vacuum evaporator at 80 °C; 100 rpm; 400 mm Hg. The product was recrystallized using methanol–distilled water, filtered using Whatmann filter paper, washed with distilled water and then dried. Yield 70%; M.P. 246-248°C.

### Synthesis of Pectin-Flurbiprofen Conjugate (PFl)

The solution of pectin in 25 mL DMF in a round bottom flask [2-(2-Fluorobiphenyl-4-yl) 1- Chloro propane ( 0.008 mole) was added slowly. Then mercuric bromide ( 0.008 mole) and yellow mercuric oxide ( 0.004 mole) were added, stirred and refluxed for 2-3 hr. The obtained suspension was filtered, dried in a rotatory vacuum evaporator at 75 $^{o}$C; 100 rpm; 300 mm Hg and recrystallized using methanol-distilled water. The product was filtered using Whatmann filter paper and dried. Yield 51%; M.P. 245-247 $^{o}$C.

### Synthesis of Inulin-Flurbiprofen Conjugate (IFl)

Inulin in minimum quantity of water was added to [2-(2-Fluorobiphenyl-4-yl) 1- Chloro propane (2g; 0.008 mole) in 25 mL DMF. Then mercuric bromide ( 0.008 moles) and yellow mercuric oxide ( 0.004 mole) were added, stirred and refluxed for 2-3 hr. The obtained suspension was filtered, dried in a rotatory vacuum evaporator at 90 $^{o}$C; 100 rpm; 400 mm Hg and recrystallized using methanol-distilled water. The product was filtered using Whatmann filter paper and dried. Yield 56%; M.P. 248-249 $^{o}$C.

### Synthesis of Chitosan-Flurbiprofen Conjugate (CFl )

The chitosan dissolved in minimum quantity of 2% glacial acetic acid, 25 mL DMF containing ( 0.008 mole) [2-(2-Fluorobiphenyl-4-yl) 1- Chloro propane was added under stirring. Then mercuric bromide ( 0.008 mole) and yellow mercuric oxide ( 0.004 mole) were also added to the solution, stirred and refluxed for 2 hr and then finally stirred at room temperature for 12 hr. The obtained suspension was filtered, dried in a rotatory vacuum evaporator at 90 $^{o}$C; 100 rpm; 400 mm Hg and recrystallized using methanol-distilled water. The product was filtered using Whatmann filter paper and dried. Yield 52%; M.P. 254-256 $^{o}$C.

### Characterization

**Thin Layer Chromatography *(TLC):*** the purity of the compound was checked by TLC using silica gel G, suitable solvent systems and detecting agent; iodine vapors

**FT-IR and $^{1}$ HNMR :** The IR spectrum of the synthesized conjugates was recorded on jasco V -530 FTIR in potassium bromide (anhydrous , IR grade).The $^{1}$ HNMR spectrum of conjugates was recorded in DMF , using a $^{1}$ HNMR Varian Mercury 300 Hz, with super conducting magnet.

### Swelling Study of Conjugates [24]

Accurately weighed amount of each of the conjugates ($W_o$) was dipped in the swelling medium SGF pH 1.2, SIF-1 pH 4.5, SIF-2 pH 6.8 and SCF pH 7.0. for 2 hr. At predetermined time intervals, the gel was removed from the swelling medium, blotted with filter paper to remove excess water from the gel surface, and the swollen hydrogels ($W_1$) was weighed. The swelling ratio (SR) is calculated according to the following equation

$$SR = \frac{W_1 - W_0}{W_0}$$

Where $W_1$- the weight of the swollen gel

$W_o$- the weight of the conjugate

### *In-Vitro* Drug Release Studies

*In-vitro* drug release studies were carried out according to Sounders and Ellenbogen (1985)[11] extraction techniques using USP dissolution test apparatus (apparatus 2). The dissolution studies were carried out in different dissolution medium (900 mL) including simulated colonic fluid (4.0%w/v), which was stirred at 100 rpm at 37±0.2 $^{o}$C.[25]

Samples were withdrawn periodically and compensated with an equal volume of fresh dissolution media. The drug content in the withdrawn samples was estimated spectrophotometrically at $\lambda_{max}$ 247 nm for conjugates of flurbiprofen.

**In-Vivo Animal Study [26]**

**Ulcerogenic Activity**

The ulcerogenic activity was determined by the Rainsford's cold stress method, which is used to determine ulcerogenic potency of a drug at a ten times higher dose. Albino rats were distributed in healthy control, standard group and test groups. Doses of each of the synthesized compounds were first calculated on equimolar basis of pure drug and then were converted into ten times higher doses. Formulation of synthesized compounds and standard drug were administered orally. After oral administration of 5 mL of the aqueous drug suspensions (at 10 times the normal dose), the animals were stressed by exposure to -15° for 1 h. The animals were placed in separate cages, to ensure equal cold exposure. After 2 hrs of drug administration, the rats were sacrificed using isoflurane anaesthesia, the stomach and duodenum were dissected out of the body along with the first 5 cm of the intestine, then rinsed with saline and the contents of the stomach were emptied. The stomach and the intestine were then excised open along the greater curvature and gently wiped clean with a swap dripped in saline. The mucosal damage was examined grossly using a magnifier. A score for the ulcer was studies similar to pyloric ligation induced ulcer model.[27]

Scoring of ulcer will be made as follows:

Normal stomach/intestine..... (0.0)
Red coloration..... (0.5)
Spot ulcer..... (1.0)
Hemorrhagic streak..... (1.5)
Ulcers..... (2.0)
Perforation..... (3.0)

Mean ulcer score for each animal will be expressed as ulcer index (UI):

UI = (UN + US + UP) x $10^{-1}$
UN = Average of number of ulcer per animal
US = Average of severity score
UP = Percentage of animal with ulcer

**Colonic edema study**

A section of inflammed colon of healthy control, colitis control, standard control and test control group on twelth day in TNBS induced animal model after treatment with flurbiprofen and its conjugates , was weighed then dried in an oven (80 $^{o}$C) for 24 hr and then reweighed to determine the wet-to-dry weight ratio, an indicator of colon edema.[27]

## RESULT AND DISCUSSION

***TLC***

Reduced compound of Flurbiprofen [2-(2-Fluorobiphenyl-4-yl)] Propenol, The purity of the compound was checked by the TLC using silica gel G, solvent system; n hexane: ethyl acetate: glacial acetic acid (6:3:1) detecting agent; iodine vapors. Only one spot was obtained $R_f$ - 0.77.

After chlorination of the [2-(2-Fluorobiphenyl-4-yl)] Propenol, The purity of the compound was checked by the TLC using silica gel G, solvent system; n hexane: ethyl acetate: glacial acetic acid (6:3:1), detecting agent; iodine vapors. Only one spot was obtained, Rf - 0.78.

The purity of Dextran – Flurbiprofen Conjugate was checked by TLC using silica gel G, solvent system; methanol: acetic acid: water (4:1:2) detecting agent; iodine vapour. Only one spot was obtained, $R_f$ - 0.69. The purity of Pectin – Flurbiprofen Conjugate was checked by TLC using silica gel G, solvent system; ethyl acetate: water (10:1), detecting agent; iodine vapors. Only one spot was obtained, $R_f$ - 0.74. The purity of Inulin – Flurbiprofen Conjugate was checked TLC using silica gel G, solvent system; ethyl acetate: water (10:1), detecting agent, iodine vapors. Only one spot was obtained, $R_f$ - 0.67. The purity of Chitosan- Flurbiprofen Conjugate was checked by TLC using silica gel G, solvent system ethyl acetate: acetic acid: water (5:1:2), detecting agent, iodine vapors. Only one spot was obtained, $R_f$ - 0.71.

**Structural elucidation**

Reduced compound of Flurbiprofen IR (KBr) spectrum: 3422.62( OH str), 3072.43(CH str aromatic), 2941.35(CH str, aliphatic), 2823.61 (CH str, aliphatic),( 1602.07, 1584.34, 1492, 1423.16,C=C str in benzene) 1071.17(C-F str) $cm^{-1}$. The disappearance of peak at 1710.21$cm^{-1}$, and appearance of peak at 3422.62 $cm^{-1}$ (m; $OH_{str}$), confirms that the carboxyl group has been reduced to methyl hydroxyl group. $^{1}$ HNMR; : δ(ppm): 1.47 ($CH_3$,3H ,d), 2.1 (CH, 1H, q), 7.2 - 7.4 (benzene,m), 4.6 (OH, 1H, s), 3.4($CH_2$, 2H, d). Chlorinated product of flurbiprofen ([2-(2-Fluorobiphenyl-4-yl) 1-Chloro propane) IR (KBr) spectrum: 3073.43(CH str aromatic), 2942.34(CH str, aliphatic), 2823.61(CH str, aliphatic), (1602.6, 1582.34, 1490.25, 1420.16 C=C str in benzene) , 1071.17 (C-F str) ,702(C-Cl str) ($cm^{-1}$). IR: 702 $cm^{-1}$($C\text{-}Cl_{str}$), confirms the formation of chloro derivative of flurbiprofen. $^{1}$ H-NMR (ppm): 1.47 ($CH_3$, 3H, d), 2.1 (CH, 1H, q), 7.2 - 7.5 (benzene,m) 3.2($CH_2$, 2H, d). IR spectrum of Dextran – Flurbiprofen Conjugate was shown some characteristic peaks at 3462.73(m; $OH_{str}$), 3038.27(CH str aromatic), 2990.22(CH str, aliphatic), 2860.35(CH str, aliphatic), (1606.81, 1590.52, 1548.80, 1487.8 C=C str in benzene) , 1140.20(glycosidic linkage str), 1071.76(C-F str) ($cm^{-1}$) IR: IR: The disappearance of Cl str peak at 702.63 $cm^{-1}$ and appearance of peak at 1140.20$cm^{-1}$ (m; characteristic peaks in glycoside); $^{1}$H NMR: 3.6 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide. IR spectrum of Pectin – Flurbiprofen Conjugate was shown some characteristic peaks at 3454.70(m; $OH_{str}$), 3014.65(CH str aromatic), 2922.07(CH str, aliphatic), 2804.87(CH str, aliphatic), 1749.39(C=O str), (1610.52, 1564.23, 1487, 1410.76 C=C str in benzene), 1116.76(glycosidic linkage str), 1071.76(C-F str) ($cm^{-1}$) IR The disappearance of Cl str peak at 702.63 $cm^{-1}$ and appearance of peak at 1116.76 $cm^{-1}$ (m; characteristic peaks in glycoside); $^{1}$H NMR: 3.40 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide.

IR (KBr) spectrum of Inulin – Flurbiprofen Conjugate was shown some characteristic peaks at: 3508.41(m; $OH_{str}$), 3014.65(CH str aromatic), 2922.07(CH str, aliphatic), 2873.31(CH str, aliphatic), (1608.53, 1595.09, 1533.37, 1440.79 C=C str in benzene), 1178.15(glycosidic linkage str) 1071.76(C-F str) ($cm^{-1}$ ) The disappearance of Cl str peak at 702.63 $cm^{-1}$ and appearance of peak at 1163.05 $cm^{-1}$ (m; characteristic peaks in glycoside); $^{1}$H NMR: 3.52 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide. IR spectrum of Chitosan- Flurbiprofen Conjugate was shown some characteristic peaks at 3538.31(m; $OH_{str}$),( 3390.31, 3308.67 NH str), 3014.65(CH str aromatic), 2922.07(CH str, aliphatic), 2849.39(CH str, aliphatic), ( 1602.81, 1523.18, 1470.79, 1407.351 C=C str in benzene),, 1193.91(glycosidic linkage str), 1071.76(C-F str) ($cm^{-1}$) IR :The disappearance of Cl str peak at 702.63 $cm^{-1}$ and appearance of peak at 1193.91 $cm^{-1}$ (m; characteristic peaks in glycoside); $^{1}$H NMR: 3.6 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide. Swelling Index:

Drug release from swellable conjugates depends on the degree of gelation, hydration, chain relaxation and erosion of the polymer. Swelling studies were performed in simulated fluids to evaluate drug release kinetics of DFl, PFl, IFl and CFl and 6.09±0.26, 4.81±0.19, 3.80±0.11and 2.81±0.08 swelling ratio were found respectively in SGF. Conjugates synthesized with dextran showed higher swelling than the conjugates with chitosan in SGF. DFl, PFl, IFl and CFl showed 8.06±0.34, 7.45±0.29, 7.02±0.34and 4.09±0.18 swelling ratio respectively in SIF II .Drug conjugates with pectin and dextran showed higher swelling than conjugates with chitosan in SIF II. DFl, PFl, IFl and CFl showed 7.19±0.32, 6.09±0.24, 5.08±0.22and 3.89±0.15 swelling ratio respectively in SIF I. Swelling studies of drug conjugates with pectin and dextran showed higher swelling than conjugates with chitosan in SIF I. Swelling studies of the drug conjugate were also performed in SCF the swelling ratio 30.62±1.11, 25.08±1.08, 27.14±1.12and 35.29±1.31were found in case DFl, PFl, IFl and CFl respectively .Drug conjugates with chitosan showed maximum swellability while conjugates with pectin showed minimum swellability in SCF.

**Table 1: Swelling Ratio of the Synthesized Conjugates in Simulated Fluids**

| Conjugates | Simulated gastric fluid | Simulated intestinal fluid I | Simulated intestinal fluid II | Simulated colonic fluid |
|---|---|---|---|---|
| DFl | 6.09±0.26 | 7.19±0.32 | 8.06±0.34 | 30.62±1.11 |
| PFl | 4.81±0.19 | 6.09±0.24 | 7.45±0.29 | 25.08±1.08 |
| IFl | 3.80±0.11 | 5.08±0.22 | 7.02±0.34 | 27.14±1.12 |
| CFl | 2.81±0.08 | 3.89±0.15 | 4.09±0.18 | 35.29±1.31 |

Values represent Mean ±SD, n=6

### *In-Vitro* Drug Release

*In-vitro* drug release studies showed drug release upto 4.26±0.03%in SGF, 12.41±0.08% in SIF and 92.72±3.33%from conjugates in SCF which confirm the stability of drug conjugates in SGF and would have more potential for colon specific delivery. The initial drug release in SGF might be due to the fact that a small number of charges present in SGF might have allowed a faster drug release as a result of higher solvent penetration into the polymeric network. This would have resulted into a faster rate of polymer hydration in acidic pH of SGF. Once the outer layer is hydrated/gelled, it acts as a barrier for the drug release and the drug then slowly diffuses out independent of pH. The drug release in SIF I and SIF II could be attributed to the fact that there might be an ion exchange phenomenon between these fluids, but release of drug from conjugates in colon is due to presence of microfloral enzymes which degrade polysaccharide as well as hydrolysis of glycosidic linkage in conjugates. The results of this study revealed that the conjugates would be suitable for colonic delivery system by the formation of conjugates of naproxen with polysaccharides.

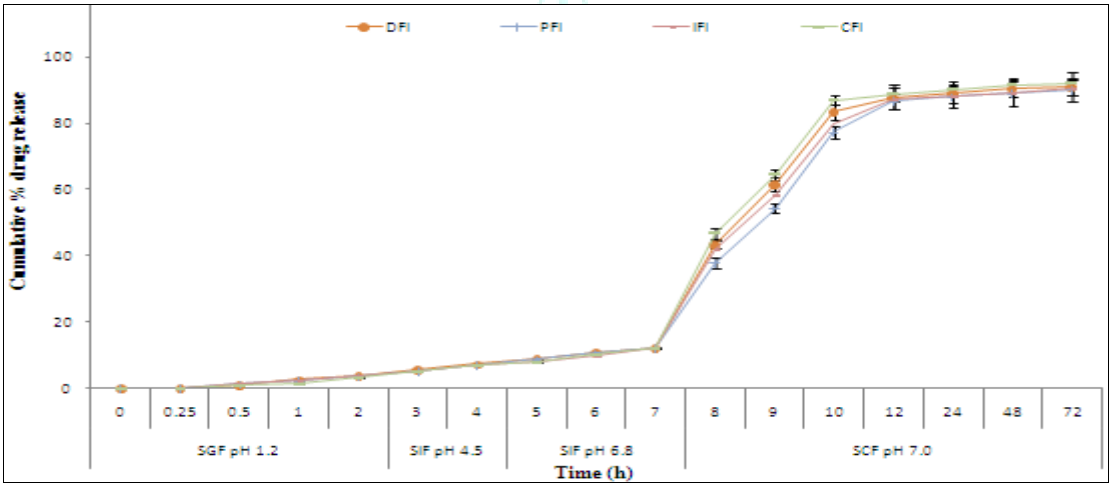


**Figure 1: Cumulative % drug release from conjugates**

### Colonic edema study

A section of affected colon was weighed, dried in an oven (80 $^{o}$C) for 24 hr and then reweighed to determine the wet-to-dry weight ratio, an indicator of colon edema .this pharmacological evaluation shows that the wet-to –dry weight ratio of animals of test groups was very close to healthy group. This study confirms better anti-inflammatory activity of conjugates as compared to parent drug .The Wet/dry weight ratio of Healthy ,Colitis control, Flurbiprofen , DFl, PFl, IFl and CFl was 4.2±0.22, 6.4±0.23, 6.1±0.12, 4.41±0.19, 5.09±0.21, 4.56±0.23and 4.28±0.17respectively.

**Table 2: Wet/Dry Weight Ratio of the Albino Rats Colon**

| S. No. | Groups | Wet/dry weight ratio |
|---|---|---|
| 1 | Healthy | 4.2±0.22 |
| 2 | Colitis control | 6.4±0.23 |
| 3 | Flurbiprofen | 6.1±0.12 |
| 4 | DFl | 4.41±0.19 |
| 5 | PFl | 5.09±0.21 |
| 6 | IFl | 4.56±0.23 |
| 7 | CFl | 4.28±0.17 |

Values represent mean± SD (n=6)

### In-vivo ulcerogenic activity

The ulcerogenic activity was determined by the Rainsford's cold stress method, which is used to determine ulcerogenic potency of a drug at a ten times higher dose. Albino rats were distributed in healthy control, standard group and test groups. Doses of each of the synthesized compounds were first calculated on equimolar basis of Flurbiprofen (10 mg /kg) and then were converted into ten times higher doses.The conjugates of drug shown very less ulcer index as compared to pure drug which were Healthy (2.12±0.57), Flurbiprofen (29.62 ±0.31), DFl (4.72±0.56), PFl (5.16±0.72), IFl (6.32±0.22) and CFl (3.21±0.51).

**Table 3: Ulcerogenic activity of drug/formulations**

| S. No. | Group | Ulcer Index |
|---|---|---|
| 1 | Healthy | 2.12±0.57 |
| 2 | Flurbiprofen | 29.62 ±0.31 |
| 3 | DFl | 4.72±0.56 |
| 4 | PFl | 5.16±0.72 |
| 5 | IFl | 6.32±0.22 |
| 6 | CFl | 3.21±0.51 |

## CONCLUSION

The ileo-colonic region of the GIT has become an important site for drug targeting and drug absorption. Colon drug delivery system provides local and systemic treatment to the patients suffering from colon infections and colon diseases. However more commonly; systems that use natural materials that degraded by colonic bacterial enzymes are used now a days for ileo-colon specificity. Different diseases such as ulcerative colitis, colon cancer, and diarrhea can be treated by using delivery of drug through colon method and by this route different drugs of different nature and molecular weight can be administered effectively This method is good for poorly absorbed drugs because colon provides long retention time and shows good absorption. By comparing the dissolution data of test formulations and standard %age drug release was predicted. It was observed that release of drug in gastric media was minimum and it was also observed that release of drug in microbial media with phosphate buffer pH 7.0 was maximum. Conclusively an appropriate dosage form of conjugate can be considered as therapeutically efficacious system for treatment of colitis, with reduced gastric intolerance.

## Conflict of interest

Authors have reported no conflict of interest.

## REFERENCES


1. Cortesi R, Ravani L, Menegatti E, Esposito E, Ronconi F, Eudragit® microparticles for the release of budesonide: a comparative study, *Indian J Pharm Sci,* 2012; 4:415-421.
2. Dubey R, Dubey R, Omrey P, Vyas SP, Jain SK, Development, and characterization of colon specific drug delivery system bearing 5-ASA and Camylofine dihydrochloride for the treatment of ulcerative colitis, *J Drug Target,* 2010; 18:589-601.
3. Sareen R, Jain N, Rajkumari A, Dhar KL, pH triggered delivery of curcumin from Eudragit-coated chitosan microspheres for inflammatory bowel disease, characterization and pharmacodynamic evaluation, *Drug Deliv,* 2016; 23:55-62.
4. Mundargi RC, Patil SA, Agnihotri SA, Aminabhavi TM, Development of polysaccharide-based colon targeted drug delivery systems for the treatment of amoebiasis, *Drug Dev Ind Pharm,* 2007; 33:255-264.
5. Kim H, Lee Y, Yoo H, Kim J, Kong H, Yoon JH, Jung Y, Kim YM, Synthesis and evaluation of sulfate conjugated metronidazole as a colon-specific prodrug of metronidazole, *J Drug Target,* 2012; 20:255-263.
6. Deo VK, Kato T, Park EY, Virus-Like Particles Displaying Recombinant Short-Chain Fragment Region and Interleukin 2 for Targeting Colon Cancer Tumors and Attracting Macrophages, *J Pharm Sci*, 2016; 105:1614-1622.
7. Rai G, Yadav AK, Jain NK, Agrawal GP, Eudragit-coated dextran microspheres of 5-fluorouracil for site-specific delivery to colon, *Drug Deliv,* 2016; 23:328-337.
8. Bansal D, Gulbake A, Tiwari J, Jain SK, Development of liposomes entrapped in alginate beads for the treatment of colorectal cancer, *Int J Biol Macromol,* 2016; 82:687-695.
9. Kato T, Yui M, Deo VK, Park EY, Development of Rous sarcoma Virus-like Particles Displaying hCC49 scFv for Specific Targeted Drug Delivery to Human Colon Carcinoma Cells, *Pharm Res*, 2015;32:3699-3707.
10. Pawar VK, Meher JG, Singh Y, Chaurasia M, Surendar Reddy B, Chourasia MK, targeting of gastrointestinal tract for amended delivery of protein/peptide therapeutics, strategies and industrial perspectives, *J Control Release,* 2014; 196:168-183
11. Yassin AE, Aodah AH, Al-Suwayeh S, Taha EI, Theophylline colon specific tablets for chronotherapeutic treatment of nocturnal asthma, *Pharm Dev Technol*, 2012; 17:712-718.
12. Mastiholimath VS, Dandagi PM, Jain SS, Gadad AP, Kulkarni AR. Time, and pH dependent colon specific, pulsatile delivery of theophylline for nocturnal asthma, *Int J Pharm,*2007; 328:49-56.
13. Sanka K, Pragada RR, Veerareddy PR, A pH-triggered delayed-release chronotherapeutic drug delivery system of aceclofenac for effective management of early morning symptoms of rheumatoid arthritis, *J Microencapsul* 2015; 32:794-803.
14. Kadiyam R, Muzib YI, Colon specific drug delivery of tramadol HCl for chronotherapeutics of arthritis. *Int J Pharm Investig*, 2015; 5:43-49.
15. Jose S, Prema MT, Chacko AJ, Thomas AC, Souto EB, Colon specific chitosan microspheres for chronotherapy of chronic stable angina. *Colloids Surf B Biointerfaces*, 2011; 83:277-283.
16. Patel P, Dhake A, Design, and development of colon specific microspheres for chronotherapy of hypertension. *J Pharm Bioallied Sci,* 2012; 4:S33-S34.
17. Ha T, Lou Z, Baek SJ, Lee SH, Tolfenamic acid downregulates β-catenin in colon cancer, *Int Immunopharmacol,* 2016; 35:287-293.
18. Hauso O, Martinsen TC, Waldum H, 5-Aminosalicylic acid, a specific drug for ulcerative colitis, *Scand J Gastroenterol*, 2015; 50:933-941.
19. Kean WF, Antal EJ, Grace EM, Cauvier H, Rischke J, Buchanan WW. The pharmacokinetics of flurbiprofen in younger and elderly patients with rheumatoid arthritis, *J Clin Pharmacol*, 1992; 32:41-48.
20. Davies NM, Clinical pharmacokinetics of flurbiprofen and its enantiomers, *Clin Pharmacokinet,* 1995; 28:100-114.
21. Binkley R W. 1998. In; Modern Carbohydrate Chemistry, Oligosaccharide synthesis, Marcel Dekker Inc., New York 297-323.
22. Excoffier G, Gagnaire D, Utille Jean-Pierre. Synthesis of reducing disaccharides of D-xylopyranose, *Carbohyd Res,* 1984; 128(2):217-226.
23. Claffey D J, Casey M F, Finan P A. Glycosylation of l,4:3,6-dianhydro-D-glucitol (isosorbide). *Carbohyd Res,* 2004; 339(14):2433-2440.
24. Friend B, Sherma J. In; Thin layer chromatography, CRC Press, U.S.A. 1999; 25:89-109.
25. Indian Pharmacopoea, Published by controller of publication under ministry of Health and Family welfare, Government of India, New Delhi 4th Eds.( 1996) A-144.
26. Maris B, Verheyden L, Van Reeth K, Samyn C, Augustijns P, Kinget R, Van den Mooter G. Synthesis and characterisation of inulin-azo hydrogels designed for colon targeting, *Int J Pharm,* 2001; 213:143–152,.
27. Nagpal D, Singh R, Gairola N, Dhaneshwar S. Mutual azo prodrug of 5 aminosalicylic acid for colon targeting. Indian journal of pharmaceutical sciences, 2006; 68(2):171-178.