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COLON TARGETED DRUG DELIVERY SYSTEM OF CELECOXIB- FORMULATION AND IN VITRO, IN VIVO EVALUATION

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ABSTRACT
Colon-targeted drug delivery systems can provide therapeutic benefits including better patient compliance and lower costs. The present investigation is aimed to design a colon specific microbially triggered system using biodegradable co-polymer mixtures. The calibration curves of Celecoxib were measured in distilled water, 0.1N HCl and phosphate buffer of pH 6.8 and 7.4 which showed good linearity. Compatibility study of pure drugs, excipients and their physical mixtures were evaluated and passed as per standards. Solubility determination was carried out in different solvents. Satisfactory results were found from evaluation of micromeritic parameters such as flow property, in-vitro dissolution study and kinetic study. Differential scanning calorimetry studies showed no incompatibility of drug with other excipients. Drug release was accelerated in the presence of rat cecal contents and indicated the degradation of polysaccharide by colonic microflora. A short-term stability study of the optimized formulation showed no significant changes in physical appearance, drug content, and in-vitro dissolution profile when stored at 40 ± 2°C/75 ± 5% RH for 3 months. The designed system can be potentially used as a carrier for colon delivery of celecoxib by regulating drug release in stomach and small intestine.

KEYWORDS: Celecoxib, Colon, Targeted drug delivery system, RP-HPLC.

INTRODUCTION
Colon targeted drug delivery systems have gained a great deal of attention as potential carriers for the treatment of colonic diseases with reduced systemic side effects and for the enhanced oral delivery of various therapeutics vulnerable to acidic and enzymatic degradation in the upper gastrointestinal tract. In recent years, the global pharmaceutical market for biologics has grown, and increasing demand for a more patient-friendly drug administration system highlights the importance of colonic drug delivery as a noninvasive delivery approach for molecules. Colon-targeted drug delivery systems can provide therapeutic benefits including better patient compliance and lower costs.

Natural polysaccharides are being extensively manipulated for the colon drug delivery systems. Nowadays, therapeutic compositions are being investigated that can effectively play a versatile role. Colon cancer is caused by a cascade of genetic mutations leading to progressively disordered local DNA replication and accelerated colonocyte replication. The progressive accumulation of multiple genetic mutations results in the transition from normal mucosa to benign adenoma to severe dysplasia to frank carcinoma (Capped, 2005). In brief, colon cancer develops in the colonic region of the lower gastrointestinal (GI) tract and usually develops slowly over a period of many years. 5-Fluorouracil (5-FU), a pyrimidine analog is one of the most extensively employed antineoplastic antimetabolite for colon cancer as well as breast cancer for six decades. It is sparingly water soluble (< 0.1 g/100 mL at 19 °C, logP ~ -0.8 and pKa ~ 8.02) drug which interferes with DNA synthesis by blocking the thymidylate synthetase conversion of deoxyuridylic acid to thymidylic acid. Effects on RNA occur especially with bolus administration. 5-FU is cell cycle phase specific (S-Phase) (www.drugbank.ca). 5-FU is well distributed into tumors, intestinal mucosa, bone marrow, liver and other tissues.

MATERIALS AND METHODS
Materials
Celecoxib was obtained as a gift sample from Vineet laboratories limited, Telangana, Hyderabad, India. HPMC was obtained as a gift sample from Kayel

Medichem Private Limited, New Delhi, India. Carbopol 71G-NF (granular grade) was obtained as gift sample from Neutron Drugs & Pharmaceuticals Private Limited, Hyderabad, Telangana, India. Xanthan gum was obtained as a gift sample from Kayel Medichem Private Limited, New Delhi, India.

Methods

Physicochemical characterization of CLX

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the drug was performed on the FTIR spectrophotometer (60MHz Varian EM 360 Perkin Elmer) using KBr pellet technique.

Differential scanning calorimetry (DSC)

The characteristic endotherm and the enthalpy of the drug were obtained using DSC technique. Samples were placed in the A1 pans sealed using hydraulic press which were heated under the nitrogen flow of (20ml/min) at a scanning rate of 10°C from 20°C to 350°C. Empty aluminium pan was used as reference. The drug-excipient compatibility studies were also carried out in the physical mixtures of various tablet excipients and polymers ((in equal proportions 1:1) using DSC (Q20 series, TA instruments, USA).

Solubility studies

The solubility of CLX in various solvents such as water, 0.1N HCl and phosphate buffer pH 6.8, phosphate buffer pH 6.8 (1% SLS) and phosphate buffer pH 7.4 was determined by shake flask method. The excess amount of the drug was added to 10 ml of the medium and stirred continuously for 72 h at 37±0.5°C until equilibration of the drug was maintained. The samples were withdrawn at different intervals (i.e. 24 h, 48 h and 72 h). The solubility of CLX in different media was determined (spectrophotometrically after filtering the samples through a 0.22µ, membrane filter) in triplicate. The UV detections were carried out at 273 nm for 0.1N HCl or water and 278 nm for phosphate buffer (pH 6.8, pH 6.8 (1% SLS), and pH 7.4)

Preparation of standard plots of 5-fu using uv-spectrophotometer

Preparation of standard plot of 5-FU in phosphate buffer (pH 6.8)

Preparation of standard plot of CLX in pH 6.8 buffer

Standard plot of CLX was prepared in pH 6.8. A stock solution of CLX (100 µg/ml) was prepared by dissolving 100 mg of accurately weighed drug in 100 ml of volumetric flask containing 1-2 ml of methanol and volume was made up to 100 ml with phosphate buffer to obtain a stock of concentration 1 mg/ml. 10 ml of this solution was further diluted to 100 ml with phosphate buffer (100 µg/ml). Further, it was serially diluted to obtain concentrations ranging from 1-100 µg/ml. The samples were then analysed spectrophotometrically at wavelength of 278 nm.

Preparation of colon targeted tablet systems of clx

Formulation development of matrix systems of CLX

Effect of fillers

The effect of the diluents (such as spray dried lactose, microcrystalline cellulose (MCC) and mixture of MCC: spray dried lactose in a ratio of 2:1 respectively) used to formulate CLX tablets were evaluated for mechanical strength and drug release properties. The composition of the tablets is shown in Table 1.

Table 1: Composition of the CLX tablets using different diluents.

Batch	CLX (mg/tablet)	Spray dried lactose (mg/tablet)	MCC (mg/tablet)
CLXM	200	-	382
CLXL	200	382	-
CLXML (2:1)	200	127.5	254.5

Boswellia gum-based matrix tablets

Matrix tablets containing 200 mg of CLX were formulated using a natural gum polysaccharide, Boswellia gum which exhibit potent anti-inflammatory activities, microcrystalline cellulose (MCC PH 102 as diluent), talc (as glidant) and magnesium stearate (as lubricant). Tablets were prepared using direct compression method. Drug, Boswellia gum and MCC were screened through #30 mesh sieve and were properly mixed. Then, talc and magnesium stearate (previously passed through #60) was added to the powder blend and were mixed thoroughly. The final blend was subjected to direct compression on multi punch (12 mm standard concave punches) 16-station rotary machine (Cadmach, India). Then, matrix tablets of average weight 600 mg were further evaluated for various parameters. Table 2 depicts the composition of the matrix tablets prepared with various concentrations of Boswellia gum.

Table 2: Composition of the matrix tablets prepared various concentrations of Boswellia gum.

Batch	CLX (mg/tablet)	Boswellia gum (mg/tablet)	MCC (mg/tablet)
BOG-60	200	360	22
BOG-50	200	300	82

Fenugreek gum-based matrix tablets

Matrix tablets containing 200 mg of CLX were formulated using another natural gum polysaccharide, fenugreek gum (which also exhibit high anti-inflammatory potential), microcrystalline cellulose (MCC PH 102 as diluent), talc (as glidant) and magnesium stearate (as lubricant). Tablets were prepared using direct compression method. CLX, fenugreek gum

powder (previously screened through #60) and MCC were screened through #30 mesh sieve and were properly mixed. Then, talc and magnesium stearate (previously passed through #60) was added to the powder blend and were mixed thoroughly. The final blend was subjected to direct compression on multi punch (12 mm standard concave punches) 16-station rotary machine (Cadmach®, India). These fenugreek matrix tablets of average weight 600 mg were further evaluated for various parameters. Table 3 indicates the composition of the matrix tablets prepared various concentrations of fenugreek gum.

Table 3: Composition of the matrix tablets prepared various concentrations of fenugreek gum.

Batch	CLX (mg/tablet)	Fenugreek gum (mg/tablet)	MCC (mg/tablet)
FNG-60	200	360	22

Carbomer-gum matrix tablets

Another matrix tablet formulations were formulated to study the suitability of the polymer blend of carbomers (e.g. Carbopol 71G-NF) and gum polysaccharides (such as Boswellia gum or fenugreek gum) for the colon specific delivery of the drug. The objective was to add an anti-inflammatory agent in the matrix as well as to impart swell ability to improve the dosage design than the single gum matrix formulations having low mechanical strength. Matrix tablets containing 200 mg of CLX, Boswellia gum or fenugreek gum, microcrystalline cellulose, talc, magnesium stearate was prepared by direct compression technique. Powder blends of drug, MCC and gum were obtained by screening (through #30 mesh sieve) and mixing (with the help of inflated bags) which was later blended with magnesium stearate and talc to lubricate and improve the flow properties of the powder. Further, the final powder blend was used for the preparation of the carbomer-gum matrix system. The compositions of carbomer-gum matrix systems are described in the Table 4.4 as given below:

Table 4: Composition of the matrix tablets prepared various concentrations of mixture of carbomer and polysaccharides.

Batch	CLX (mg/tablet)	Boswellia gum (mg/tablet)	Fenugreek gum (mg/tablet)	MCC (mg/tablet)	Carbopol 71G (mg/tablet)
BOG-10	200	60	-	82	240
BOG-40	200	240	-	82	60
FNGC-41	200	-	240	82	60

Polyethylene oxide (PEO) matrix tablets

Another matrix formulation system was developed containing polyethylene oxide (PEO) to retard the drug release in the initial hours. Matrix tablets comprising 200 mg of CLX were formulated by direct compression making use of varied ratios of PEO polymer and microcrystalline cellulose as indicated the Table 4 Powder blend of the given matrix system was prepared by screening of drug, PEO and MCC through #30 mesh sieve and further were mixed thoroughly with magnesium stearate and talc to lubricate and improve the flow properties of the powder. The final powder blend was used for making tablets on rotary multi punch (16 station) machine on standard 12 mm concave punches.

Table 5: Composition of matrix tablets containing different ratio of PEO.

Batch	CLX (mg/tablet)	PEO (mg/tablet)	MCC (mg/tablet)
CLXPEO1	200	60	322
CLXPEO2	200	120	262
CLXPEO3	200	180	202
CLXPEO4	200	240	142

Hydroxy propyl methyl cellulose (HPMC) based matrix tablets

Another matrix system was also developed using different grades of hydroxy propyl methyl cellulose (HPMC) polymer. Matrix tablets were made by first mixing all the excipients (MCC, HPMC K4M or HPMC K100M respectively) along with the drug (200 mg) thoroughly followed by sieving to obtain a uniform blend. Finally, the lubricant and glidant were added and the blend was further mixed for ten minutes and then compressed into tablets on multi punch rotary machine using 12.0 mm concave punches. The powder blend equivalent to 600 mg was weighed and compressed individually. Table 6 shows the composition of core of matrix tablets prepared with a polymer blend containing two different polymers in varying ratios.

Table 6: Composition of matrix tablets containing different Grades and Ratios of hydroxy propyl methyl cellulose HPMC.

Batch	CLX (mg/ tablet)	HPMC K4M (mg/ tablet)	HPMC K100M (mg/ tablet)	MCC (mg/ tablet)
CHPK41	200	60	-	322
CHPK42	200	120	-	262
CHPK43	200	180	-	202
CHPK44	200	240	-	142
CHPK45	200	300	-	82
CHPK1001	200	-	60	322
CHPK1002	200	-	120	262
CHPK1003	200	-	180	202
CHPK1004	200	-	240	142
CHPK1005	200	-	300	82

Enteric (Mixed film) coated delayed release systems of CLX

Fabrication of core tablets of CLX

The core tablets containing 200 mg of CLX were prepared using direct compression technique. The composition of the core tablets included microcrystalline cellulose (MCC 102), talc (glidant), and magnesium stearate as shown in Table 7. Briefly, Drug and MCC were screened through # 22 mesh sieve and were mixed properly using inflated polybags for ten minutes. To the above blend, talc and magnesium stearate were added and blended thoroughly. Further, the final blend was used to prepare the core tablets of CLX weighing 600 mg on the Cadmach sixteen station rotary tablet machine using 12 mm standard concave punches.

Table 7: Composition of core tablets of CLX.

Batch	CLX (mg/tablet)	MCC (PH 102) (mg/tablet)	Talc (mg/tablet)	Mg stearate (mg/tablet)
CLX	200	382	12	6

Preparation of coating solutions

Ethyl cellulose: Eudragit ®S-100 coating solution

The coating dispersion of concentration 6% w/v of ethyl cellulose and Eudragit®S-100 in two varied ratios (1:1 and 3:1 respectively) was obtained by dissolving the both coating polymers in isopropyl alcohol: ethanol (3:1) mixture. Dibutyl phthalate (at concentration of 12.0% w/w of polymer, specific density ~1.042g/ml) was used as plasticizer. The coating solution was continuously vortexed using rotary mixer at uniform speed of 2000 rpm for 30 minutes until clear solution was obtained.

Ethyl cellulose: Eudragit ®L-100 (3:1) coating solution

Another coating solution containing ethyl cellulose and Eudragit®L-100 of 6% w/v concentration in a ratio of 3:1 was obtained by dissolving the two polymers one after other in IPA: ethanol (3:1) mixture. Dibutyl phthalate as plasticizer was used as plasticizer at a concentration 12.5% w/w of total polymer weight. A clear uniform coating solution was formed after vortex mixing of 30 minutes at 2000 rpm.

Coating of core tablets of CLX

The core tablets of CLX were coated with the above-mentioned coating solutions at various coat levels using pan coating method and dried with the help of dryer with an inlet air temperature of 40-50°C. The coating process was continued till the desired level of coating thickness was achieved. The coating thickness was measured based on coat weight with respect to the weight of tablets. Table 8 depicts the list of various formulation, compositions, and percent coat weight gain of the CLX tablets.

Table 8: Batch codes, ratios of the coating mixtures used for the mixed film enteric coated delayed release Systems and Coat weight gain levels.

Batch	Ethyl cellulose	Eudragit ®S-100	Eudragit ®L-100	Coat weight (%W/W)
CLXS-I	1	1	-	1.991
CLXS-II	1	1	-	3.831
CLXS-III	1	1	-	5.170
CLXS-IA	3	1	-	1.769
CLXS-IB	3	1	-	3.702
CLXS-IC	3	1	-	5.663
CLXL-I	3	-	1	1.887
CLXL-II	3	-	1	4.377
CLXL-III	3	-	1	5.938

Formulation of starch containing batches

Starch (10%w/w) containing core tablets containing 200 mg of CLX were prepared using direct compression technique. The composition of the core tablets included microcrystalline cellulose (MCC 102), starch (10% w/w) talc (glidant), and magnesium stearate as shown in Table 9. Briefly, Drug, starch and MCC were screened through # 22 mesh sieve and were mixed properly using inflated polybags for ten minutes. To the above blend, talc and magnesium stearate were added and blended thoroughly. Further, the final blend was used to prepare the starch core tablets of CLX weighing 600 mg on the Cadmach® sixteen station rotary tablet machine using 12 mm standard concave punches.

Table 9: Composition of starch containing core tablets of CLX.

Batch	CLX (mg/tablet)	Starch (mg/tablet)	MCC (PH 102) (mg/tablet)	Talc (mg/tablet)	Mg stearate (mg/tablet)
CLXS	200	60	322	12	6

Table 10: Batch codes, ratios of the coating mixtures used for the mixed film enteric coated delayed release systems with coat weight gain levels.

Batch	Ethyl cellulose	Eudragit ®S-100	Eudragit ®L-100	Coat weight (%W/W)
CLXSS-I	3	1	-	1.234
CLXSS-II	3	1	-	1.548
CLXSS-III	3	1	-	2.650
CLXSL-I	3	-	1	1.010
CLXSL-II	3	-	1	1.616
CLXSL-III	3	-	1	2.551

RESULTS AND DISCUSSION

Fourier transform infrared (ftir) spectroscopy

Infrared spectroscopy is comprehensively employed in pharmaceutical analysis for fingerprint identification of a drug molecule and for the proof of its structural validation. Infrared absorption spectroscopy, especially when measured by means of the Fourier transform method (FTIR), is a powerful technique for the physical characterization of pharmaceutical solids which pose different IR characteristics, and is sensitive to crystalline form changes. Since, it is inherently based on the molecular vibrations; it has the advantage of being sensitive to functional group changes in low or non-crystalline materials.

The IR spectrum of the CLX was studied as Nujol oil using (60 MHZ Varian EM 360, Perkin Elmer, USA) IR spectrophotometer which revealed characteristic absorption bands at 3334.92cm^{-1}, 3332.89cm^{-1},1512.64cm^{-1}, 1450.31cm^{-1}, 1373.82cm^{-1}, 1345.41cm^{-1}, 1342.67 cm^{-1}, 1273.96 cm^{-1},1260.21 cm^{-1} and 1150.78 cm^{-1} which were observed to be approximately like the reported literature values. The band at 3334.92 cm^{-1} indicated characteristic -OH stretching vibration frequency. The absorption band at 3332.89 cm^{-1} corresponds to NH2 groups. The absorption band at 1512.64 cm^{-1} corresponds to -C=C- aromatic stretching vibration frequency. The band at 1450.31 cm^{-1} indicated characteristic Methyl --CH3 bending vibration frequency. The other absorption band at 1373.82 cm^{-1}was due to the CN functional group. Furthermore, the band at 1345.41 cm^{-1} indicated characteristic -C-O-C- stretching vibration frequency for cyclic ether link. The other absorption band at 1342.67 cm^{-1}was due to the-S=O sulphoxide asymmetric stretching vibration frequency. Further the other absorption band at 1273.96 cm^{-1} was due to the CF functional group. The absorption band at 1260.21 cm^{-1} corresponds to C-N stretching vibration frequency. The absorption band at 1150.78 cm^{-1} corresponds to -S=O sulphoxide symmetric stretching vibration frequency. The FTIR spectrum of the drug is illustrated in the Fig.1

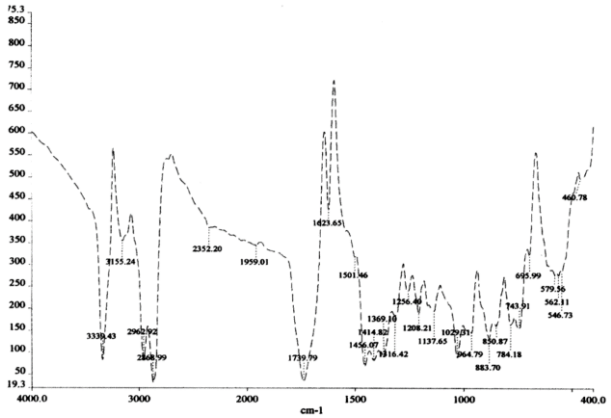

Fig. 1: FTIR Spectra of CLX.

Solubility studies

Solubility analysis is one of the most important physicochemical properties studied during pharmaceutical preformulation. Accurate solubility data are essential to ensure the robustness of the finished product and to determine, if an adequate amount of drug is available for absorption in-vivo. If a compound has a low aqueous solubility, it may be subject to dissolution rate-limited or solubility-limited absorption within the gastrointestinal (GI) residence time. The importance of solubility, in biopharmaceutical terms, is highlighted by its use in the biopharmaceutics classification system (BCS) described by Amidon *et al.* (1995). Using the shake flask technique, the solubility of CLX was observed in various solvents (such as water, 0.1N HCl and pH 6.8 phosphate buffer, pH 6.8 phosphate buffer (1% SLS) and pH 7.4 phosphate buffer) at different intervals (i.e. 24 h, 48 h and 72 h) for 72 hours in triplicate. The solubility studies performed on CLX indicated pH dependent solubility. The solubility data revealed that the CLX is hydrophobic in nature or poorly soluble in water and 0.1 N HCl, however, at higher pH (i.e. alkaline) the drug was found to be highly soluble.

Table 11: Solubility analysis of celecoxib observed at different pH solvent media.

S. No.	Solvent media	Solubility (mg/ml)
1	Water	0.563
2	0.1NHCl	0.394
3	pH 6.8 phosphate buffer	31.84
4	pH 6.8 phosphate buffer (1% SLS)	45.50
5	pH 7.4 phosphate buffer	23.16

Differential scanning colorimetry of clx

Differential scanning calorimetry (DSC) is a widely used technique within the pharmaceutical industry, because the range of phase transitions it can measure usually allows near comprehensive physical characterization of a new active principle during preformulation. This technique helps to evaluate physical properties of drugs, as well as to study compatibility and stability of the components of pharmaceutical preparations.

The DSC thermogram of CLX is indicated in the Fig. 2 Sharp single endothermic transition characterized by an onset of temperature of 150.71°C, a temperature maximum of 151.62°C and an enthalpy of fusion of 64.08 J/g was observed. The values were found to be in good agreement with the literature values.

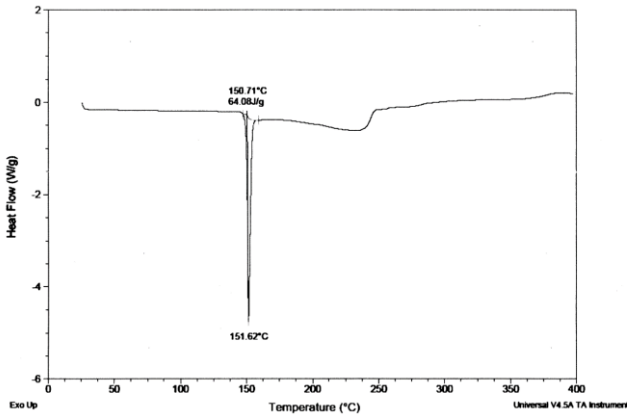

Figure 2: DSC of CLX.

Table 12: Absorbances of celecoxib against different concentrations in pH 6.8phosphate buffer.

Concentration (µg/ml)	Absorbance
0	0
2	0.143
4	0.301
6	0.43
8	0.583
10	0.713
12	0.85
14	0.989

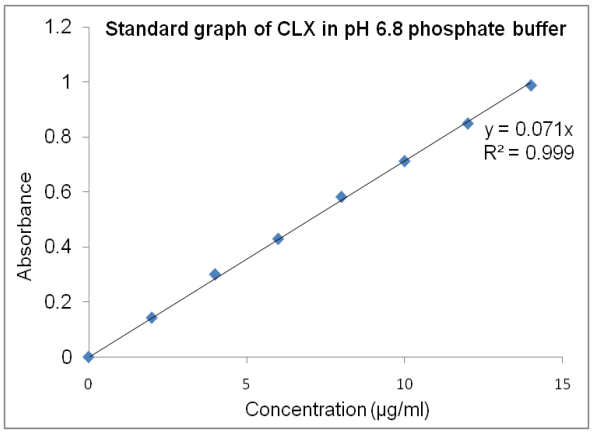

Fig. 3: Standard graph of CLX in 6.8 Phosphate buffer.

Evaluation of colon targeted compositions of clx

Physical evaluation

The pre-formulation stage of any product development such as tablet, constitutes evaluation of physical parameters (Tablet shape, tablet dimensions) and physical testing (Weight, thickness; hardness, friability, drug content etc.). The quantitative evaluation and assessment of a tablet's chemical, physical and bioavailability properties are significant to monitor product quality. Thus, these properties are imperative since chemical breakdown or interactions between tablet components may alter the physical tablet properties, and greatly affect the bioavailability of the tablet system. Hence, the physical evaluation of various optimized formulation batches was performed. The CLX tablet batches prepared for colon targeted drug delivery followed I.P. compendial test limits. All the batches exhibited average weight in the range of 598.74 to 605.01mg and passed the uniformity of weight as per l.P (I.P., 2007).

The tablet hardness of CLX batches prepared with fillers MCC, lactose and mixture of MCC: Lactose (CLXM, CLXL and CLXML respectively) were found in the range 9.6 to 12.8 kg/cm2. MCC as a filler exhibited best compressibility and tablet hardness among the other excipients or combinations for the formulation of CLX tablets. CLX tablets batches containing gums/ gum combinations (BOG-50, BOG-60, FNG-60, BOGC-10, BOGC-40, and FNGC-41) as controlled release polymers showed relatively lesser hardness in comparison to the plain CLX tablets. The hardness was observed in the range of 7.2 to 10.8 kg/cm. Boswellia gum containing tablet batches (BOG-60 and BOG-50) showed average hardness of 9.6 kg/cm2 irrespective of change in the gum concentration in the tablet composition. Tablet batches prepared with fenugreek gum showed comparatively low hardness value (i.e. 7.8 kg/cm2) with respect to Boswellia gum batches. The tablet batches containing mixture of granular grade Carbopol®71G and Boswellia gum polymers (batches- BOGC-10, BOGC-40) exhibited 7.7 and 7.2 kg/cm2 of hardness respectively. However, tablet batch (FNGC-41) containing mixture of fenugreek gum and Carbopol®71G showed 10.8kg/cm2 of tablet hardness. Thus, it was observed that Boswellia gum-CLX tablets showed slight change in hardness on increasing the concentration of Carbopol®71G polymer in the tablet matrix whereas fenugreek gum-Carbopol®71G showed the vice-versa effect. Moreover, tablet batches prepared with polyethylene oxide (Polyox WSR-303, granular grade) showed a very good direct compressibility and produced the tablets of good mechanical strength in the range between 5.8 to 6.9kg/cm2. Tablet batches prepared using HPMC grades K4M and K100M grade showed very high mechanical strength along with very low friability. HPMC K4M batches (CHPK41, CHPK42, CHPK43, CHPK44, and CHPK45) and HPMC K100M (CHPK1001, CHPK1002, CHPK1003, CHPK1004 and CHPK1005) exhibited tablet hardness in the range of 10.9 to 11.9kg/cm2 and 8.7 to 11.3 kg/cm2 respectively. Further, HPMC (K4M or K100M) containing tablet batches showed significantly high hardness in comparison to all other prepared batches of CLX for colonic delivery. It was also observed that the average hardness of each batch increased with the rise in the concentration of HPMC K4M/K100M in the tablet matrix, this property of the HPMC may be attributed to the high binding of the HPMC matrix and the plastic deformation with a low degree of fragmentation under the compression pressure.

The thickness of the tablet was found to be almost uniform in all formulations CLXM to CT (Table 5.5). The average thickness was found to be in the range of 6.45 to 6.63mm. The friability test for all the formulations were done as per the standard procedure of I.P. The results of the friability test were tabulated in Table 13. The data indicates that the percent friability was less than 1% in all formulations.

Moreover, all the CLX batches showed percentage of drug content (% assay) in the range of 97.57% to 103%. Hence, it is concluded that all the formulations were evaluated for physical parameters which followed the desired acceptable limits as per I.P. (I.P., 2007) i.e. ± 5%.

Table 13: Physical evaluation of various CLX tablets formulated for colon delivery.

Batch	Hardness (Kg/cm2) (mean±S.D) *	Average weight (mg) (mean±S.D.) *	Thickness (mm) (mean±S.D) *	Friability (%)	Drug Assay (%)
CLXM	12.8±0.34	599.73±2.15	6.63±0.08	0.06	100.6± 2.89
CLXL	9.6±0.35	599.01±2.55	6.55±0.09	0.11	100.3±3.99
CLXML	12.3±0.38	599.99±2.87	6.50±0.03	0.08	99.6±1.32
BOG-60	9.6±0.46	600.63±3.21	6.57±0.04	0.13	98.9±2.37
BOG-50	9.6±0.23	600.88±3.71	6.58±0.05	0.21	99.4±1.39
FNG-60	7.8±0.45	598.37±2.15	6.50±0.02	0.22	99.9±1.86
BOGC-10	7.7±0.39	599.93±3.23	6.51±0.03	0.31	100.7±2.90
BOGC-40	7.2±0.41	600.71±3.48	6.52±0.01	0.16	101.8±0.68
FNGC-41	10.8±0.60	598.77±3.01	6.52±0.02	0.19	98.6±2.35
CLXPEO1	6.9±0.81	600.89±3.20	6.47±0.06	0.37	101.8±3.55
CLXPEO2	5.8±0.38	602.46±4.88	6.46±0.06	0.34	99.7±1.87
CLXPEO3	6.6±0.32	602.18±4.37	6.51±0.03	0.30	100.0±1.29
CLXPEO4	6.9±0.51	601.66±4.50	6.49±0.04	0.24	99.0±2.28
CHPK41	10.9±0.70	599.87±2.46	6.52±0.02	0.04	103.0±1.39
CHPK42	11.8±0.63	602.59±2.52	6.50±0.03	0.08	99.81±2.99
CHPK43	11.9±0.82	600.79±3.38	6.52±0.03	0.11	101.0±3.48
CHPK44	11.7±0.47	605.01±5.14	6.51±0.03	0.06	97.57±0.44
CHPK45	11.1±0.49	601.75±3.30	6.50±0.05	0.09	101.85±2.76
CHPK1001	9.9±0.64	601.40±3.44	6.48±0.04	0.17	100.9±3.01
CHPK1002	10.1±0.65	602.24±3.81	6.52±0.03	0.18	99.01±1.32
CHPK1003	9.5±0.73	599.46±3.56	6.51±0.02	0.19	101.30±1.79
CHPK1004	8.7±0.40	601.99±2.62	6.45±0.08	0.13	99.21±2.88
CHPK1005	11.3±1.24	603.76±2.80	6.49±0.04	0.12	101.78±1.83
CT	7.9±0.29	598.74±3.24	6.51±0.02	0.14	99.91±1.89

Dissolution studies of colon targeted drug delivery systems of CLX

The prime challenge in the initial development of colon-specific drug delivery systems was to establish an appropriate dissolution testing method to evaluate the designed system in-vitro. Various alternative approaches had been investigated previously to simulate the colonic conditions and to make the feasibility of dissolution method bio-relevant. To analyse the dissolution characteristics of a diverse colon-specific drug delivery system, proper understanding of the colon's hydrodynamics, colonic motility, and disease pattern is required. The targeting of the drug specifically to the colonic site requires lag period of 5-6 h (2 h of gastric emptying time + 3 h of small intestine transit time). United States Pharmacopeia (USP) dissolution testing procedure for the evaluation of delayed release dosage forms devises a simple, convenient and bio relevant method in different buffers (known as method A). In this method, colonic dosage forms designed with a lag phase of 5-6 h would be tested in 0.1 N HCl for 2 h and the rest of the dissolution phase will be carried out in phosphate buffer pH 6.8. Various systems and dosage forms for the specific targeting of the CLX at the colonic site were formulated as mentioned below:

Fabrication of matrix tablets of CLX

CLX matrix tablets with different fillers

To design a colon targeted tablet dosage form, the selection of excipient or filler was carried to obtain tablets of good mechanical strength, dissolution characteristics and tablet properties (such as shape, surface area, porosity, disintegration, hygroscopicity, palatability etc.). The effect of excipients such as MCC, lactose and its combination ratio (2:1) was evaluated by preparing plain tablets of CLX using various fillers. The in-vitro release was determined for tablets prepared using three different fillers. From the dissolution profile, it was found that initial release for 2hr was only 2.99±0.89% for the CLXL tablets in comparison to CLXM (19.23±2.98%) and CLXML (14.23±8.21%) tablets respectively (Table 14).

The dissolution profiles showed batch CLXM had the fastest release; batch CLXL showed the slowest release; and batch CLXML exhibited the release in-between (Table 5.6). During the dissolution, CLXML tablets swelled, broke apart and exhibited burst release at 3 h (Fig. 5.22). These tablets also revealed early complete release at 5 h. However, batch CLXM exhibited swelling with burst release at 3 h, but showed complete release at 8 h. On the other hand, lactose tablet was intact during the dissolution studies. This tablet batch exhibited initially slower release (2.99±0.89%), but after 3 h, it showed slow drug release and the release was observed at 24 h. MCC is the most frequently used directly compressible tablet excipient having unique fibrous structure which offers excellent compressibility and high capacity to accommodate co-processed ingredients. Moreover, MCC showed better compression properties

and tablet integrity. However, lactose produced tablets which were brittle and of low mechanical strength. At the same time, the drug release was faster from these tablets. Hence, MCC was chosen as filler for the preparation of plain core tablets of CLX.

Table 14: Cumulative percent release of CLX tablets prepared from different fillers.

Time (hr)	Cumulative percent released ± SD (n=3)		
	CLXM	CLXL	CLXML
0	0	0	0
0.5	8.01±1.48	1.23±0.18	6.01±2.54
1	15.01±2.35	2.01±0.35	9.12±3.99
2	19.23±2.98	2.99±0.89	14.23±8.21
3	80.03±0.68	24.12±0.33	72.05±9.98
4	93.45±3.01	43.22±1.70	92.63±3.25
5	96.00±0.56	59.99±3.03	100.21±0.84
6	98.91±1.89	72.11±1.26	-
8	100.63±0.60	96.30±2.98	-
10	-	98.67±0.34	-
12	-	99.96±0.87	-
24	-	100.74±0.79	-

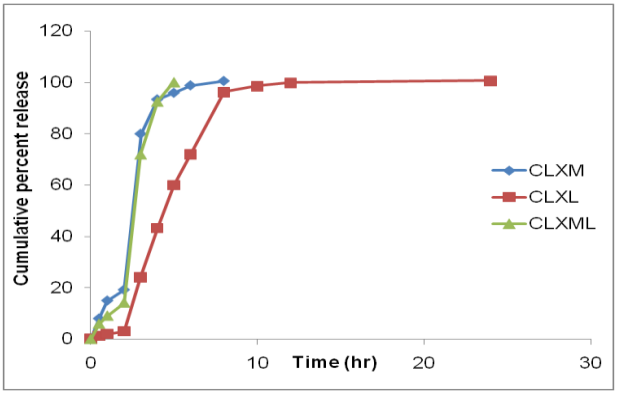

Figure 4: Cumulative percent release versus time for formulations CLXM, CLXL and CLXML (mean ± S.D.; n=3).

Boswellia gum matrix tablets of CLX

Recently, natural polymers such as gum polysaccharides have attracted the attention of pharmaceutical technologists for the design of oral controlled drug delivery systems due to number of advantages over synthetic polymers because of their cost effectiveness, easy availability, biodegradability, non-toxicity, and broad regulatory acceptance. Natural polysaccharides like Boswellia gum tends to hydrate and swell in contact with the aqueous media thereby regulate the release of drug component from the dosage forms. Boswellia gum is basically a resin exudate obtained from Boswellia serrata roxburghii and other species of Boswellia. Further, the resin contains mainly a resin acid like Boswellic acid which exhibit anti-inflammatory properties. Boswellia gum has been exploited as drug release retardant. Thus, Boswellia gum was used in the matrix tablet formulation to retard the initial drug release using direct compression method. Initially higher concentrations (batches BOG-60 (with 60%w/w of total weight) and BOG-50 (50%w/w of total weight) of Boswellia gum were used to evaluate the effect on drug release and tablet properties. It was found that batch BOG-60 and BOG-50 showed 10.04±0.63% and 15.98±2.29% of drug release during initial 2 hr release respectively. Further, more than 50% of the drug was released at the 5-hr interval in both the batches. The high amount of drug release during initial 5 hr may be attributed to the hydrophilic nature of the gum which led to the increased porosity and solubilization of the gum. Moreover, incomplete release was observed at 24 hr i.e. 75.36±2.21% for batch BOG-60 and 78.77±2.84% for batch BOG-50 respectively. Hence, these higher concentrations of the Boswellia gum were not effective enough to retard the drug release during initial 5 h as shown in the Table 15 and Fig 5

Table 15: Cumulative percent release from Boswellia gum-based matrix tablets of CLX.

Time (hr)	Cumulative percent released ± SD (n=3)	
	BOG-60	BOG-50
0	0	0
0.5	2.68±0.73	5.11±0.18

1	6.01±0.54	9.01±0.33
2	10.04±0.63	15.98±2.29
3	48.66±4.01	43.67±2.75
4	51.89±3.68	48.54±0.98
5	54.01±3.88	52.74±2.68
6	56.12±3.55	54.98±1.47
8	59.99±4.48	60.31±0.99
10	64.11±3.12	64.21±4.32
12	67.43±2.19	67.54±3.02
24	75.36±2.21	78.77±2.84

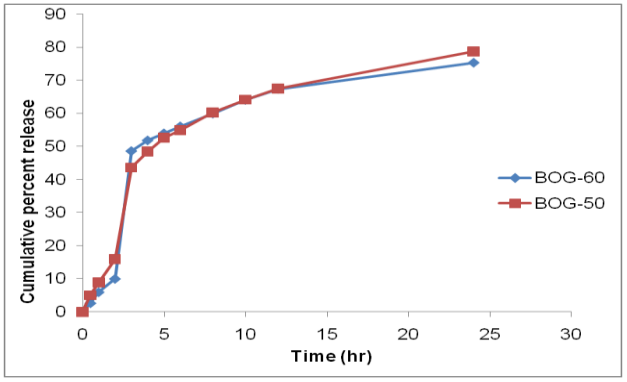

Figure 5: Cumulative percent release versus time for formulations BOG-60 and BOG-50 (mean ± S.D.; n=3).

Fenugreek gum matrix tablets of CLX

Fenugreek gum is another natural gum polysaccharide which forms a viscous tacky mass when exposed to fluids and swells up to provide the control release properties. Thus, a batch containing 60% w/w (of total tablet weight) of fenugreek gum was used to develop a matrix tablet formulation to retard the release of CLX. However, the matrix tablet could not retard the drug release and exhibited rapid release i.e. 38.12±2.01% in initial 2 hr. Afterwards, the tablet showed burst release and gave complete drug release at 3 h (Table 16 and Fig. 6). Tablets containing fenugreek gum failed in sustaining or retarding the CLX release in matrix formulation and further optimization using some synthetic release retardants (such as Carbopol ®71G) was carried out.

Table 16: Cumulative percent release from fenugreek gum-based matrix tablets of CLX.

Time (hr)	Cumulative percent released ± SD (n=3)
	FNG-60
0	0
0.5	34.32±0.72
1	36.21±1.33
2	38.12±2.01
3	100.56±1.26

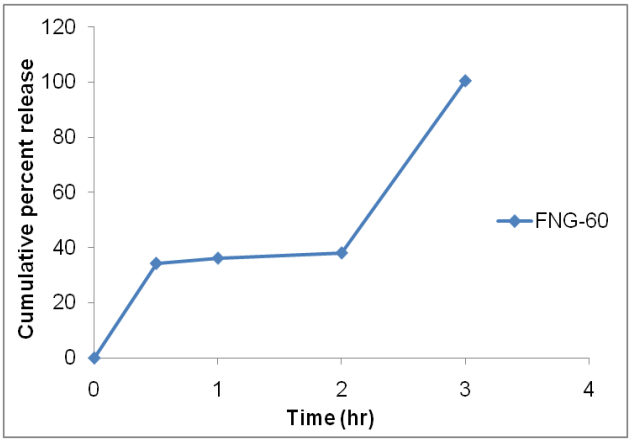

Figure 6: Cumulative percent release versus time for formulation, FNG-60 (mean ± S.D.; n=3).

Carbomer-gum based matrix tablets of CLX

It was observed that both the natural gums, Boswellia and fenugreek gum as individual polymer components were not able to control the drug release even at higher concentrations. Therefore, a fraction of the gum component was replaced by directly compressible, granular grade, pH dependent swelling carbomer, Carbopol®71GNF, widely used for the dissolution rate controlling properties.

Carbomers exhibit high water sorption property and swells up to 1000 times of their original volume and 10 times their original diameter to form a gel when exposed to a pH of 4.0 to 6.0. When carbomer incorporating tablets are placed in contact with dissolution media, the external surface of the tablet becomes hydrated, swells, and forms a gel layer that further controls the release of the drug from the tablets.

Mixture of Boswellia gum and Carbopol®71G (Batches BOGC-10, BOGC-40) were formulated containing 10%, 40%, w/w (of total tablet weight) of Boswellia gum with 40%, 10% w/w (of total tablet weight) of Carbopol®71G respectively whereas Batch FNGC-41 was also prepared containing 40% w/w (of total tablet weight) of fenugreek gum with 10% w/w (of total tablet weight) of Carbopol®71G respectively. The dissolution studies on batch BOGC-10 (10% w/w of Boswellia gum and 40% w/w of Carbopol®71G) indicated 90.93±2.14% of drug release in the initial 3 hr as shown in Table 5.9 and Fig. 5.25. The batch showed premature release and could not be able to retard the drug release. Thus, batch with higher concentrations of the gum, BOGC-40 (40% w/w of Boswellia gum and 10% w/w of Carbopol®71G) was designed to prevent initial high drug release. Batch BOGC-40 showed 76.32±3.15% of CLX release in first 3 hr (Fig. 7) and followed constant release thereafter from the swelled matrix up to 95.99±4.75% at 24 hr (Table 5.9). It was observed that even higher amounts of Boswellia gum in the carbomer matrix did not help in preventing the drug exposure during the basic pH environment. Furthermore, batch FNGC-41 (40% w/w of fenugreek gum and 10% w/w of Carbopol ®71G) was also developed to evaluate the effect of fenugreek gum in combination with carbomer in delaying the drug release from matrix tablet formulations. The batch indicated premature release and drug was completely released in 3 hr. Hence, the matrix tablets containing swelling hydrophilic polymers (gums and carbomers) were not suitable to check initial CLX release.

Table 17: Cumulative percent release from carbomer-gum based matrix tablets of CLX.

Time (hr)	Cumulative percent released ± SD (n=3)		
	BOGC-10	BOGC-40	FNGC-41
0	0	0	0
0.5	25.30±2.35	17.23±0.91	24.98±1.41
1	30.01±0.89	20.11±2.23	27.42±1.47
2	32.63±0.76	26.01±2.32	32.78±2.35
3	90.93±2.14	76.32±3.15	100.93±2.16
4	94.02±1.44	82.78±4.00	-
5	95.76±1.13	88.20±5.20	-
6	94.91±3.45	89.18±2.83	-
8	94.44±0.48	89.55±5.42	-
10	98.48±4.48	93.38±3.81	-
12	97.81±2.00	94.84±4.58	-
24	96.98±0.77	95.99±4.75	-

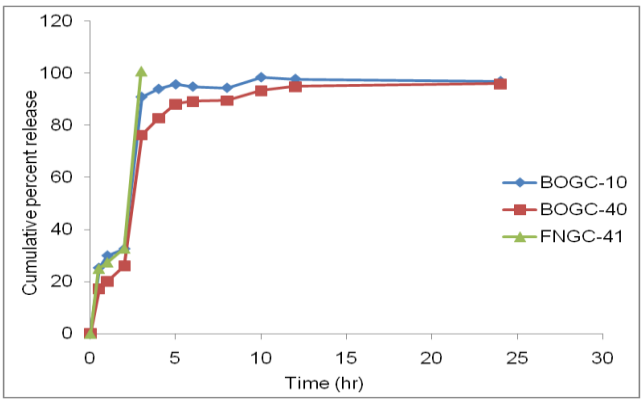

Figure 7: Cumulative percent release versus time for formulations BOGC-10, BOGC-40, and FNGC-41 (mean ± S.D.; n=3).

Table 18: Comparative profile of various drug release models, best fit models for the CLX tablet batches suitable for colon drug delivery.

Batch	Release models										
	Zero order		First order		Higuchi		Korsmeyer-Peppas		Hixon-Crowell		Best fit model
	k_0	R_0	k_1	R_1	k_h	R_h	n	R_k	k_s	R_s	
CLXPEO3	3.93	0.988	-0.085	0.864	12.78	0.751	1.59	0.978	-0.1550	0.8286	Zero order
CLXPEO4	3.76	0.997	-0.085	0.735	11.73	0.735	1.72	0.950	-0.1592	0.8273	Zero order
CLXSS-III	4.98	0.874	-0.091	0.963	16.95	0.714	1.95	0.983	-0.1827	0.7592	Peppas
CLXSL-III	4.86	0.899	-0.089	0.945	15.86	0.698	1.71	0.974	-0.1780	0.7866	Peppas

CONCLUSION

The aim of the present study was to develop novel formulation systems using conventional chemotherapeutic agents and chemo preventive NSAIDs, specifically targeted at the colonic site for the management of colon cancer.

Clinical intervention of NSAIDs as chemo preventive agents have been commenced after evidence-based data from number of case-control, prospective, and clinical studies of NSAIDs. Among the diverse class of NSAIDs, CLX was major drug candidate which was chosen for the present study.

After the selection of NSAID, pre formulation studies on CLX were commenced. Different parameters such as UV spectrum, FTIR, solubility, DSC analysis was studied. UV spectrums were performed in various solvent media viz. 0.1 N HCl, methanol, and phosphate buffer (pH 6.8) with λmax values of drug obtained at 220, 236 and 265 nm, respectively. Further, FTIR analysis of CLX was performed which showed characteristic absorption bands at 3334.92cm^{-1}, 3332.89cm^{-1},1512.64cm^{-1}, 1450.31cm^{-1}, 1373.82cm^{-1}, 1345.41cm^{-1}, 1342.67 cm^{-1}, 1273.96 cm^{-1},1260.21 cm^{-1} and 1150.78 cm^{-1} which were found to be in concordance with the literature values.

Further, solubility studies in water, 0.1 N HC1 and phosphate buffer (pH 6.8) showed pH dependent solubility of CLX.

Characteristic peak of CLX at 150.71°C was obtained in the DSC thermograms which were found to be in accordance with the literature reports. No physical or chemical incompatibility observed between the drug and different excipients/polymers.

Various approaches were utilized for the design and development of colon specific delivery systems of CLX. Before developing the colonic targeted system, effect of fillers on the rate of CLX release from the dosage form was evaluated. Among the three fillers (i.e. MCC, lactose and combination of MCC and lactose) used, MCC was chosen for the preparation of core tablets for the further studies.

In the first approach, system was prepared by embedding of CLX in the tablet gum polysaccharide matrix (Boswellia gum or fenugreek gum) alone or in combination with a release retardant (Carbopol®71G). Another approach used for colon targeting was based on mixed film coating of the core tablets. Multiparticulate systems in the form of pellets were also formulated using different release retardants such as natural gums, polymers, and coating polymers. These pellets were developed for CLX using extrusion spheronization technique.

To achieve a successful colon targeted system, a lag phase of 5-6 h is required along with little or no release in the initial hours of the dissolution study. Matrix tablets of CLX were fabricated by embedding the drug in Boswellia gum matrix. Initially the batches containing 60% and 50% of the Boswellia gum were prepared and evaluated for the dissolution studies. Both the formulations were unable to retard the premature CLX release after 5 h of dissolution. Similarly, batch containing 60% of fenugreek gum was also prepared and studied. These batches were also failed to retain the CLX from matrix after 5 h of the dissolution study. Hence, further optimization of batches was done by adding a release retarding polymer (Carbopol®71G) to retard the initial premature release from the matrix. Batches BOGC-10 (containing 40% of Carbopol®71G and 10% of Boswellia gum), BOGC-40 (containing 10% of Carbopol®71G and 40% of Boswellia gum), FNGC-41 (containing 40% of fenugreek gum and 10% of Carbopol®71G) were prepared respectively. All the batches prematurely released more than 75% after 3 h of the dissolution study. Thus, these batches were failed to achieve the successful targeting of CLX.

REFERENCES

1. Aaltonen, L.A., The multistep process of colon carcinogenesis. Cytokines Mol. Ther, 1996; 2: 111-114.
2. Adachi, T., Tajima, Y., Kuroki, T., Mishima, T., Kitasato, A., Tsuneoka, N., Kanematsu, T., Chemo preventive effects of a selective cyclooxygenase-2 inhibitor (etodolac) on chemically induced intraductal papillary carcinoma of the pancreas in hamsters. Carcinogenesis, 2008; 29: 830-833.
3. Adebamowo, C.A., Adeyi, O., Pyatt, R., Prior, T.W., Chadwick, R.B., de la Chapelle, A., Case report on hereditary non-polyposis colon cancer (HNPCC) in Nigeria. Afr. J. Med. Med. Sci, 2000; 29: 71-73.

4. Adekunle, O.O., Lawani, J.A., Clinical aspects and management of carcinoma of the rectum in Nigerians. East African Med. J, 1982; 59: 206-213.
5. Adeyemo, D., Imtiaz, F., Toffa, S.L., Wickremasinghe, R.G., Winslet, M., Antioxidants enhance the susceptibility of colon carcinoma cells to 5-Fluorouracil by augmenting the induction of the bax protein. Cancer Fett, 2001; 164: 77-84.
6. Agarwal, B., Bhendwal, S., Halmos, B., Moss, S.F., Ramey, W.G., Holt, P.R., Fovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. Clin. Cancer Res, 1999; 5: 2223-2229.
7. Aggarwal, B.B., Nuclear factor-kappa B: The enemy within. Cancer Cell, 2004; 6: 203- 208.
8. Aggarwal, B.B., Kumar, A., Bharti, A.C., Anticancer" potential of curcumin: preclinical and clinical studies. Anticancer Res, 2003; 23: 363-398.
9. Aggarwal, B.B., Shishodiab, S., Sandura, S.K., Pandeya, M.K., Sethia, G., Inflammation and cancer: How hot is the link? Biochemical Pharmacol, 2006; 72: 1605-1621.
10. Albengres, E., Pinquier, J.L., Riant, P., Bree, F., Urien, S., Barre, J., Tillement, J.P, Pharmacological criteria for risk-benefit evaluation of NSAIDs. Scand. J. Rheumatol, 1988; 17: 3-15.
11. Alberici, P., The Adenoma-Carcinoma Sequence in Colorectal Cancer: Scratching the Surface, 2007. Erasmus University Rotterdam, (available from http://hdl.handle.net/1765/9785).
12. Alberts, D.S., Ritenbaugh, C., Story, J.A., Aickin, M., Rees-McGee, S., Buller, M.K., Atwood, J., Phelps, J., Ramanujam, P.S., Bellapravalu, S., Patel, J., Bextinger, L., Clark, F., Randomized, double-blind, placebo-controlled study of effect of wheat bran fibre and calcium on faecal bile acids in patients with resected adenomatous colon polyps. J. Natl. Cancer Inst, 1996; 88: 81-92.
13. American Cancer Society. Colorectal Cancer Facts & Figures 2011-2013. Atlanta: American Cancer Society, 2011
14. American Joint Committee on Cancer. Cancer staging manual, 2003; 5: 83-90.
15. Andre, T., Boni, C., Navarro, M., Tabemero, J., Hickish, T., Topham, C., Bonetti, A., Clingan, P., Bridgewater, J., Rivera, F., de Gramont, A., Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J. Clin. Oncol, 2009; 27: 3109-3116.
16. Andre, T., Colin, P., Louvet, C., Gamelin, E., Bouche, O., Achille, E., Colbert, N., Boaziz, C., Piedbois, P., Tubiana-Mathieu, N., Boutan-Laroze, A., Flesch, M., Buyse, M., de Gramont, A., Semimonthly versus monthly regimen of fluorouracil and leucovorin administered for 24 or 36 weeks as adjuvant therapy in stage II and III colon cancer: results of a randomized trial. J. Clin. Oncol, 2003; 21: 2896-2903.
17. Andre, T., Quinaux, E., Louvet, C., Colin, P., Gamelin, E., Bouche, O., Achille, E., Piedbois, P., Tubiana-Mathieu, N., Boutan-Laroze, A., Flesch, M., Lledo, G., Raoul, Y., Debrix, I., Buyse, M., de Gramont, A, 2005. Updated results at 6 years of the GERCOR C96.1 phase III study comparing LV5FU2 to monthly 5FU leucovorin (mFufol) as adjuvant treatment for Dukes B2 and C colon cancer patients- ASCO Annual Meeting Proceedings. J. Clin. Oncol, 2005; 23: 16S, (Abstract 3522).
18. Andre, T., Toumigand, C., Mineur, L., Fellague-Chebra, R., Flesch, M., Mabro, M., Hebbar, M., Postel V.S., Bidard, F.C., Louvet, C., de Gramont, A., Phase II study of an optimized 5-fluorouracil-oxaliplatin strategy (OPTIMOX2) with celecoxib in metastatic colorectal cancer: A GERCOR study. Ann. Oncol, 2007; 18: 77-81.
19. Arber, N., Levin, B., Chemoprevention of colorectal cancer: ready for routine use, Curr. Top. Med. Chem, 2005; 5: 517-525.
20. Ashktorab, H., Dawkins, F.W., Mohamed, R., Larbi, D., Smoot, D.T., Apoptosis induced by aspirin and 5-Fluorouracil in human colonic adenocarcinoma cells. Dig. Dis. Sci, 2005; 50: 1025-1032.
21. Ayakawa, S., Shibamoto, Y., Sugie, C., Ito, M., Ogino, H., Tomita, N., Kumagai, M., Murakami, H., Sawa, H., Antitumor effects of a cyclooxygenase-2 inhibitor, meloxicam, alone and in combination with radiation and/or 5- fluorouracil in cultured tumor cells. Mol. Med. Rep, 2009; 2: 621-625.
22. Bach, S.P., Williamson, S.E., Marshman, E., Kumar, S., O'Dwyer, S.T., Potten, C.S., Watson, A.J., The antioxidant n-acetylcysteine increases 5-fluorouracil activity against colorectal cancer xenografts in nude mice. J. Gastrointest. Surg, 2001; 5: 91-97.
23. Bardou, M., Barkun, A., Martel, M., Effect of statin therapy on colorectal cancer. Gut, 2010; 59: 1572-1585.
24. Barni, S., Lissoni, P., Cazzaniga, M., Ardizzoia, A., Meregalli, S., Fossati, V., Fumagalli, L., Brivio, F., Tancini, G., A randomized study of low-dose subcutaneous interleukin-2 plus melatonin versus supportive care alone in metastatic colorectal cancer patients progressing under 5-fluorouracil and folates. Oncology, 1992; 52: 243-245.
25. Barni, S., Lissoni, P., Cazzaniga, M., Ardizzoia, A., Paolorossi, F., Brivio, F., Perego, M., Tancini, G., Conti, A., Maestroni, G., Neuroimmunotherapy with subcutaneous low-dose interleukin-2 and the pineal hormone melatonin as a second-line treatment in metastatic colorectal carcinoma. Tumori, 1992; 78: 383-387.
26. Bar-Yehuda, S., Madi, L., Silberman, D., Gery, S., Shkapenuk, M., Fishman, P., CF101, an agonist to the A3 adenosine receptor, enhances the chemotherapeutic effect of 5-fluorouracil in a colon carcinoma murine model. Neoplasia, 2005; 7: 85-90.
27. Becerra, C.R., Frenkel, E.P., Ashfaq, R., Gaynor, R.B., Increased toxicity and lack of efficacy of rofecoxib in combination with chemotherapy for

treatment of metastatic colorectal cancer: A phase II study. Int. J. Cancer, 2003; 105: 868-872.
28. Bongaerts, B.W., de Goeij, A.F., van den Brandt, P.A., Weijenberg, M.P., Alcohol and the risk of colon and rectal cancer with mutations in the K-ras gene. Alcohol, 2006; 38: 147-154.
29. Cai, M., Wang, G.B., Tao, K.X., Cai, C.X., Effect of enhancing chemotherapy sensitivity by combining siRNA recombinant expression vector targeting surviving gene with 5-FU on human colon cancer cells. Chin. Med. Sci. J, 2009; 24: 97-101.
30. Calviello, G., Di Nicuolo, F., Serini, S., Piccioni, E., Boninsegna, A., Maggiano, N., Ranelletti, F.O., Palozza, P., Docosahexanoic acid enhances the susceptibility of human colorectal cancer cells to 5-fluorouracil. Cancer Chemother. Pharmacol, 2005; 55: 12-20.
31. Carnesecchi, S., Langley, K., Exinger, F., Gosse, F., Raul, F., Geraniol, a component of plant essential oils, sensitizes human colonic cancer cells to 5-fluorouracil treatment. J. Pharmacol. Exp. Ther, 2002; 301: 625-630.
32. Chan, A.T., Giovannucci, E.L., Meyerhardt, J.A., Schemhammer, E.S., Curhan, G.C., Fuchs, C.S., Long-term use of aspirin and non-steroidal anti-inflammatory drugs and risk of colorectal cancer. J. Am. Med. Assoc, 2005; 294: 914-923.
33. Chan, A.T., Ogino, S., Fuchs, C.S., Aspirin use and survival after diagnosis of colorectal cancer. J. Am. Med. Assoc, 2009; 302: 649-658.
34. Chan, J.Y., Tan, B.K., Lee, S.C., Scutellarin sensitizes drug-evoked colon cancer cell apoptosis through enhanced caspase-6 activation. Anticancer Res, 2009; 29: 3043- 3047.
35. DuBois, R.N., Giardiello, F.M., Smalley, W.E., Nonsteroidal anti-inflammatory drugs, eicosanoids, and colorectal cancer prevention. Gastroenterol. Clin. North Am, 1996; 25: 773-791.
36. DuBois, R.N., Gupta, R., Brockman, J., Reddy, B.S., Krakow, S.L., Lazar, M.A., The nuclear eicosanoid receptor, PPAR-gamma, is aberrantly expressed in colonic cancers. Carcinogenesis, 1998; 19: 49-53.
37. Dukes, C.E., The classification of cancer of the rectum. J. Pathol. Bacteriol, 1923; 35: 323-332.
38. Dupertuis, Y.M., Meguid, M.M., Pichard, C., Colon cancer therapy: new perspectives of nutritional manipulations using polyunsaturated fatty acids. Curr. Opin. Clin. Nutr. Metab Care, 2007; 10: 427-432.
39. Felth, J., Rickardson, L., Rosen, J., Wickstrom, M., Fryknas, M., Lindskog, M., Bohlin, L., Gullbo, J., Cytotoxic effects of cardiac glycosides on colon cancer cells, alone and in combination with standard chemotherapeutic drugs. J. Nat. Prod, 2009; 72: 1969-1974.
40. Fernando, N., Yu, D., Morse, M., Blobe, G., Odugwu, L., Crews, J., Polito, A., Honeycutt, W., Franklin, A., Hurwitz, H., A phase II study of oxaliplatin, capecitabine and bevacizumab in the treatment of metastatic colorectal cancer. Proc. Am. Soc. Clin. Oncol, 2005; 24: 3556.
41. Findenig, G., Mader, R.M., Fritzer-Szekeres, M., Steger, G.G., Jaeger, W., Szekeres, T., Modulation of 5-fluorouracil resistance in human colon tumor cell lines by azidothymidine. Oncol. Res, 1996; 8: 189-196.
42. Fisher, G.A., Kuo, T., Cho, C.D., Halsey, J., Jambalos, C.N., Shwartz, E.J., Robert, R.V., Advani, R.H., Wakelee, H.A., Sikic, B.I., A phase II study of gefitinib in combination with FOLFOX-4 (IFOX) in patients with metastatic colorectal cancer. Proc. Am. Soc. Clin. Oncol, 2004; 23: 249, (Abstract 3514).
43. Flis, S., Gnyszka, A., Flis, K., Splawinski, J., MS275 enhances cytotoxicity induced by 5-fluorouracil in the colorectal cancer cells. Eur. J. Pharmacol, 2010; 627: 26-32.