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SYNTHESIS AND CHARACTERIZATION OF INDOMETHACIN KONJAC GLUCOMANN COMPLEX FOR COLON TARGETTING

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Abstract

The current aim of our research work is to couple Indomethacin with Konjac glucomann through glycosidic linkage for colon targetting.We synthesized Indomethacin KGM Complex and examine the effect of enzyme β-Glucosidase on the release characteristics of Indomethacin and KGM in the gastrointestinal contents of rats. By using this approach drug (Indomethacin) can be targeted in the colon to treat the colitis .The Indomethacin KGM glycosidic linkage did not release drug in acidic environment of stomach ,but when drug reaches to colon the enzyme β-Glucosidase acts on glycosidic bond and release the drug . By using this approach drug can be targeted directly to colon. The complex was evaluated for its color, solubility, R_f value, melting point and IR analysis. It was further subjected for evaluating its colon targetting property by in-vitro method using rat fecal matter.The drug was also subjected to cytotoxic studies which shows that the complex was non toxic even at more than 200µgm/ml.

Keywords:

Coltis, KGM, NSAID, β-Glycosidase, MTT, Colitis

Introduction

Oral colon-specific delivery system is used for the treatment of various diseases of colon such as ulcerative colitis, IBS, colorectal cancer [1–3], from this approach the high local concentration of drug can be achieved and side effects can be minimized.

Glucomannan is a water-soluble polysaccharide that is considered a dietary fiber. It is a hemicellulose component in the cell walls of some plant species.

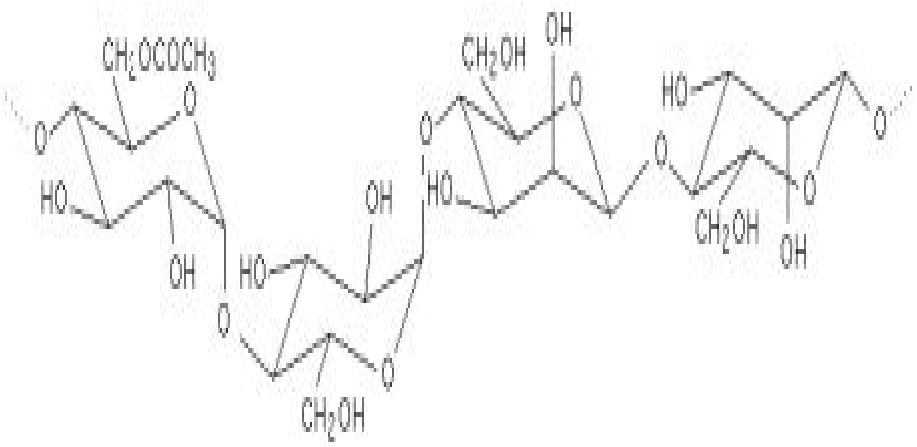
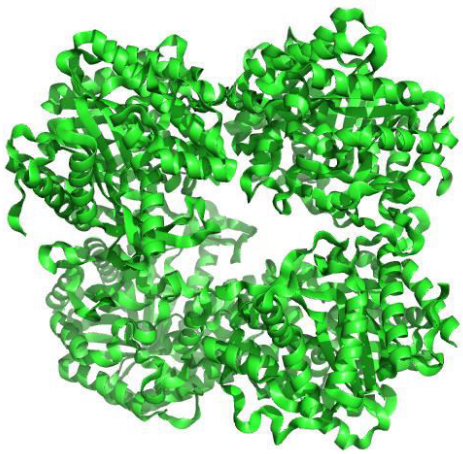


Figure1: Structure of Konjac glucomann.

KonJac Glucomann is a food additive used as an emulsifier and thickener. Glucomannan is mainly a straight-chain polymer, with a small amount of branching. The component sugars are β-(1→4)-linked D-mannose and D-glucose.[4] . Glucomannan is a soluble fiber, and as such, has been investigated for the treatment of constipation. Glucomannan may relieve constipation by decreasing fecal transit time.[5]. Glucomannan has demonstrated statistically significant improvements in the total cholesterol of obese patients.[6]. Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling.Intrarectal administration of indomethacin was associated with decreased severity of inflammation and decreased prostaglandin E2 content of the colonic mucosa.[7]

Glycosidic linkage has been used as tools to deliver the drugs especially to the colon. The glycoside bond remain intact in the physiological environment of stomach and small intestine but once the dosage form enters the colon, the enzyme β-Glucosidase act on the colon and break the bond which releases the drug into colon.



The structure of beta-glucosidase A from bacterium Clostridium cellulovorans.[8]

SCHEME

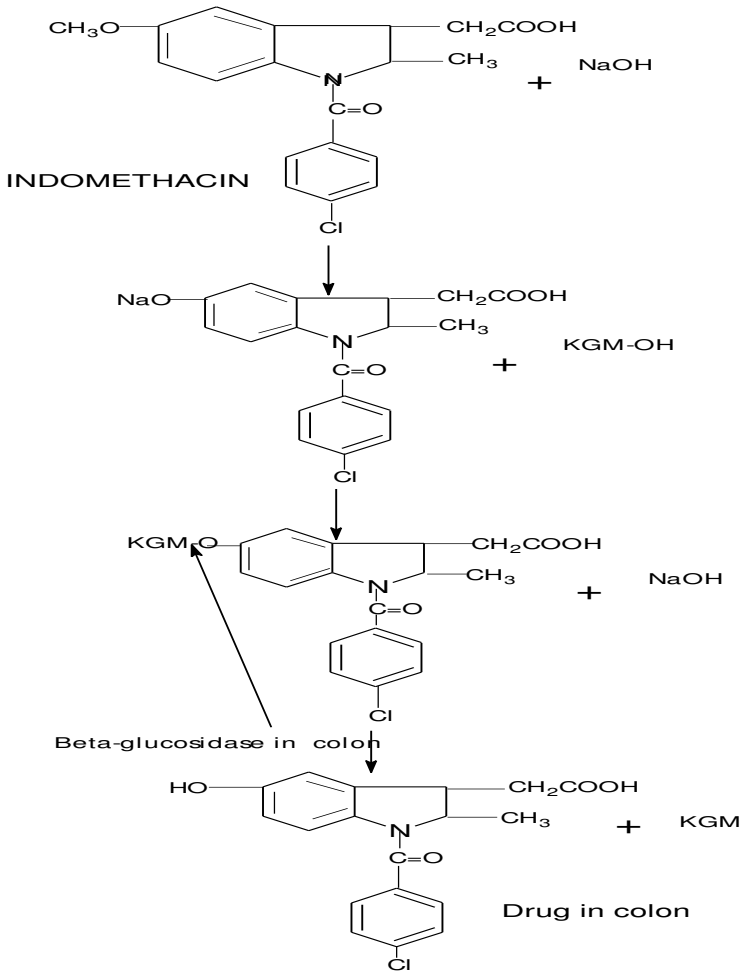


Fig 2: Indomethacin KGM complex

Experimental Methods

Indomethacin (0.5 g) was dissolved in 30 mL NaOH solution (pH=12) at room temperature for 4 hours, then konjac glucomannan (0.5 g) was added into the solution under continuous stirring. The dispersion was mixed for 8 hours to allow maximum swelling of KGM. The mixture was heated to 60ºC and incubated for 24 hours. After reaction, the compound was washed several times with double distilled water to remove the unreacted indomethacin, KGM and other soluble agents. Then the compounds were dried under 60ºC to constant weight. The TLC was taken by using toluene, ethylacetoacetate and formic acid (5:4:1) at different time interval and visualization of spots was done by using iodine chamber. The obtained product was subjected for physicochemical properties like m.p., colour, and solubility. The glycosidic linkage formation was confirmed by IR spectral studies. The derivative is further subjected for in vitro release using a rat fecal material and phosphate buffer.

Analytical methods

IR Spectra was taken on a Shimadzu Spectrophotometer. [1]. Melting and decomposition points were conducted in a melting point apparatus. The IR spectrum of the synthesized compound was obtained from Laureate Institute.

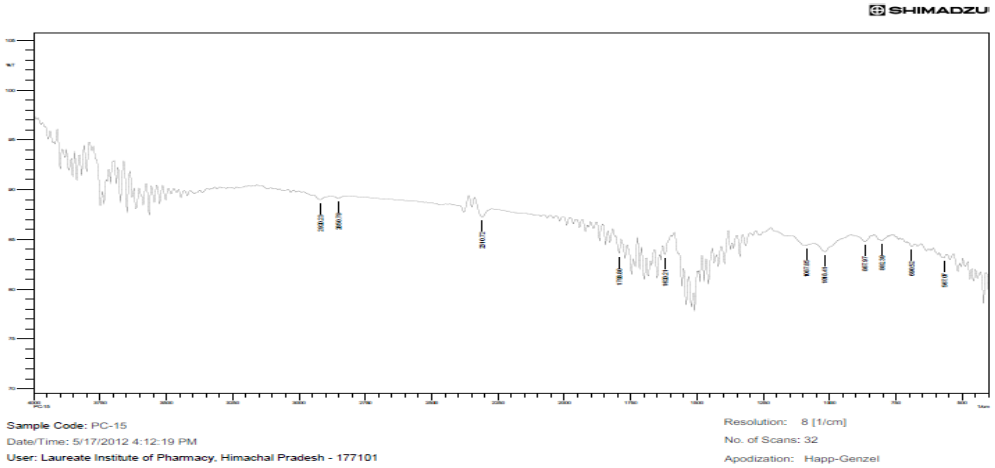


Fig 3: IR spectea of Indomethacin KGM complex

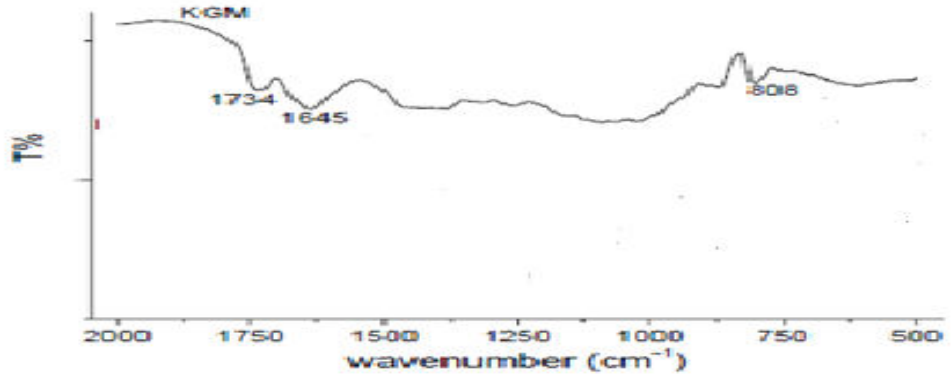


Fig 4: IR spectra of KGM

Acute Toxicity Study

The azo adduct was evaluated for acute toxicity study. The protocol was approved by the Institutional Animal Ethical Committee. OECD 423 guide-lines were followed in the procedures. Two groups of 6 albino rats, one for test and other for control, were used for the study. The study was performed by administering the Indomethacin KGM Complex at 2g/kg body weight for the test group animals. The acute toxicity study was evaluated for a period of 14 days, changes in the skin colouration, observing body weight, corneal reflex, behavioural patterns, and convulsions and compared with the control group animals [9] .

In vitro release study

The derivative is further subjected for in vitro release using a rat fecal material using phosphate buffer of Ph 7.4. Shimadzu 1800 UV spectrophotometer was used for this purpose .1 gm of rat fecal material was taken in 6 test tubes, 1 ml of drug solution (10 microgram/ml) was added in each test tube. Then 5ml of phosphate buffer was added in each test tube and incubate it for half hour at 37 ^{0} C for different interval of time. Filter the solution and for analysis, the aliquots were removed from the test tubes at different time intervals and absorbance was estimated directly on double beam UV-spectrophotometer at 320 nm for indomethacin. (Jung *et al.*, 2000)[10].

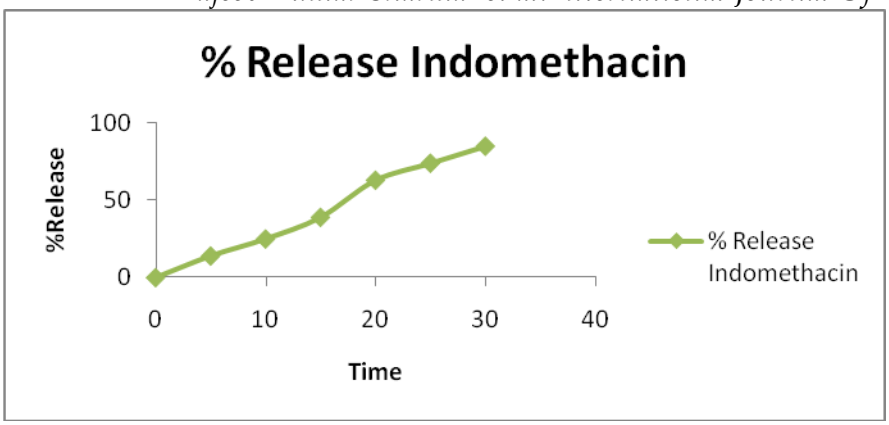
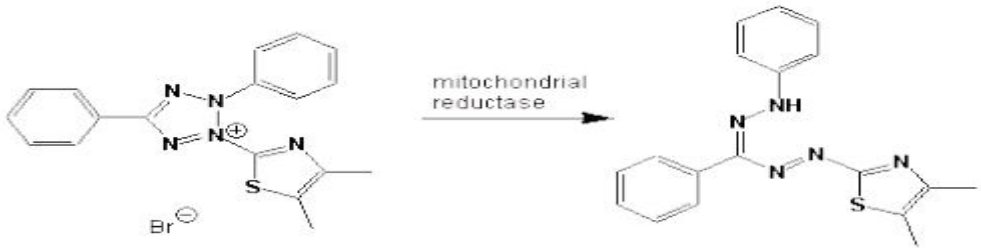


Fig 5: Percentage release of Indomethacin from Indomethcin KGM complex.

Cytotoxicity Studies-Cytotoxic studies was performed in Department of Pharmacology Manipal College of Pharmaceutical Sciences by the below mentioned principle.



This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg.isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. Following were the result of acute toxicity study. Doxorubicin was used as reference compound.

					Cell Line	**HCT-116**	**Humam colon cancer cell line**						
					Time point of treatment 48hrs								
Compound Name	Conc (µg/ml)	Absorbance at 540nm			% Cell Death			% Cell Viability			Mean Cell Viability	IC50 (ug/ml)	SEM
Indomethacin KGMcomplex	25	0.181	0.187	0.195	20.3	17.6	14.1	79.7	82.4	85.9	**82.7**	>200	1.8
	50	0.171	0.187	0.176	24.7	17.6	22.5	75.3	82.4	77.5	**78.4**		2.1
	100	0.16	0.173	0.181	29.5	23.8	20.3	70.5	76.2	79.7	**75.5**		2.7
	200	0.156	0.154	0.169	31.3	32.2	25.6	68.7	67.8	74.4	**70.3**		2.1

DOXO RUBICIN	0.05	0.205	0.214	0.21	9.7	5.7	7.5	90.3	94.3	92.5	**92.4**	**4.5**	1.1
	0.5	0.18	0.145	0.13	20.7	36.1	42.7	79.3	63.9	57.3	**66.8**		6.5
	5	0.094	0.088	0.099	58.6	61.2	56.4	41.4	38.8	43.6	**41.3**		1.4
	50	0.079	0.099	0.067	65.2	56.4	70.5	34.8	43.6	29.5	**36.0**		4.1

Results and discussion

The structure of indomethacin and KGM was characterised by IR spectroscopy .As compared with the spectrum of KGM the peak at 1734 cm^{-1} disappeared and new peaks appeared .This result showed that the crosslinking occurred between KGM and Indomethacin.IR (KBr): 2920.23 (Methylene),2850.79(-COOH)(1786.08),1085.5(C-N Stret.) 802.39(P-Substituted benzene) m.p-235^{0}C, %Yield -75%,R_f-0.72,Colour-Pale Yellow. Solubility- Soluble in phosphate buffer ph-7.4, Insoluble in dil. hydrochloric acid. The invitro release study of Indomethacin KGM Complex shows that the complex shows the good colon specificity with the release rate of approx. 85% and the drug is released after 30 min(Figure 9).No sign of acute toxic effects were observed in animals. Our cytotoxic studies reveals that Indomethacin KGM complex shows the IC50 value >200µgm/ml when compared to doxorubicin with IC50 value 4.5 µgm/ml.So this cleary indicates that Indomethacin KGM complex was non toxic even at more than 200µgm/ml .The same complex when entered into colon region is going to release indomethacin .From the cytotoxic studies it was found that the product is safe for use to colon for the treatment of Colitis.

Conclusion

Our research study result reveals it is having significant colon specificity hence this method is feasible for preparing colon targeted delivery and this complex is used for treating various diseases of colon. The conclusion was drawn that this method is so beneficial, economic and patient compatible for targeting drug to colon region in effective manner.

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