Development and evaluation of bioflexy films phoenix dactylifera with topiramate by Translabial drug delivery system

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Abstract: Formulation and evaluation of nanosized Topiramate loaded bio-flexy films using novel biopolymer isolated from phoenix dactylifera for epilepsy treatment, by using Translabial route. Translabial drug delivery system is an attractive approach for drug delivery system possess advantages as a bypass of the first-pass metabolism, prevent from digestive enzymes and rapid action of suitable drugs. The isolated biopolymer was subjected to various physicochemical characterization procedures for analyzing the mucoadhesive and mucoretentive properties. The mucoadhesive and mucoretentive properties of isolated biopolymer were analysed using the shear stress method and the MS mucoretentibility method. Methods: Formulations containing nanosized Topiramate: phoenix dactylifera biopolymer (in ratios of 1:0.5, 1:1; 1:3, 1:5, 1:6, 1:10) (FGO1-FGO6) were prepared by solvent casting method.

INTRODUCTION

Epilepsy, a chronic neurological disorder occurs due to excess of excitatory neurotransmitter discharges in brain. Every year 2.4 million people are diagnosed with epilepsy. 70% of Epilepsy patients are responsive to Antiepileptic medications. Antiepileptic drug, Topiramate possesses half-life of 19-30 hr; bioavailability: of 80%; protein binding of 13-17%; water solubility of 9.8 mg/L. It is used for Partial Onset and Generalized Onset Seizures. It augments the activity of neurotransmitter Gamma Amino Butyrate (GABA) at subtypes of GABAA Receptor. Soft palate is part of oral mucosa, constitutes back of roof of mouth. Soft palatal drug delivery provides sustained and controlled drug delivery. It has non-keratinized histology no bone, abundant blood and nerve supply, drug directly reaches systemic circulation, non-invasive, non-mobile with high mucoretention ability, afford high bioavailability, lower doses, offers a Novel mucoadhesive Drug Delivery Platform for Brain targeting. It is a promising area for systemic delivery of orally inefficient drugs, potent peptide and protein drug molecules. Trigeminal nerve directly connects soft palate to brain. Thus, drug in Nanosized form directly reaches into brain through inter and intraneural route. Since it lacks taste buds, bitter tasting drugs can be administered by this route. The soft palatal mucosa possesses unique inbuilt characteristics of not interfering with patient's regular routine activities of talking, eating, drinking, non-interference of tongue It is innervated by Mandibular branch of trigeminal nerve, Greater palatine nerve, Nasopalatine nerve, Lesser palatine nerve, Motor nerves, Glosso pharyngeal nerve, as well as Greater Palatine branch of Maxillary Artery, Ascending Palatine branch of Facial Artery, Middle Meningeal artery, Ascending pharyngeal artery, Accessory Meningeal artery. It has Thickness: 158-224 μm, pH: 7.34±0.38, Blood flow: 0.89 mL/ min/cm, Surface area: 200 cm2 2,3 In this research work, an inert, biodegradable cost effective biopolymer bio-excipient isolated from phoenix dactylifera. It contains Minerals like Calcium-28%, Iron-121%, Manganese-120%, Magnesium-79%, Phosphorus, Potassium-38%, Zinc-51%, Phytic Acid, Carbohydrates-30%, Protein-36.49%, Dietary Fiber-37%,Total Fat-20%, Omega-3 fatty acid, Omega- 6 fatty acid, B Vitamins, including Folate - 94%, Vitamin C-7%, Vitamin E-6%, Vitamin K-45%. Bio-flexy films were prepared by economical method of solvent casting. Various evaluation parameters were performed for screening of prepared Bio-flexy films formulations.

MATERIALS AND METHODS

Topiramate drug was obtained as a gift sample from Zaneka Pharmaceuticals., Haridwar. Phoenix dactylifera was procured from the local market, Dehradun. HPMC and sodium CMC were purchased from Merck Specialties Pvt. Ltd., Mumbai. Other

ingredients and double distilled water are of analytical grade

ISOLATION OF BIOPOLYMER FROM PHOENIX DACTYLIFERA:

Method for isolation of biopolymer from the pulp of Phoenix dactylifera. The Phoenix dactylifera (1000g) fruit was taken then fruit pulp was minced with double distilled water and filtered and treated with dimethyl ketone and refrigerated for 10hours.Then centrifuged at 3000 rpm for 20 minutes and the biopolymer was collected and dried in desiccator for 20 hours. The biopolymer was passed through mesh size =120 to get uniform particles.

PHYSICOCHEMICAL CHARACTERIZATION OF ISOLATED BIOMATERIAL:

the isolated bio-material was characterized for physicochemical parameters such as odour, colour, melting point, solubility along with chemical tests.

(a) texture, (b) colour, (c) odour were examined physically.
(d) Colour changing point: determined by capillary method by melting point apparatus. the bio-polymer was kept in a capillary tube and it was fitted in a melting point apparatus. temperature was determined by thermometer.
(e) solubility: determined in chloroform, methanol, distilled water, acetone.
(f) Test for carbohydrates: Molisch reagent test: 2 ml of biopolymer solution (0.1gm dissolved in 2 ml of distilled water) was taken in a test tube. added 2 drops of molisch reagent (solution of α-naphthol in 95% ethanol). concentrated sulphuric acid (2 ml) was taken in a test tube and biopolymer solution was gradually poured over it leading to the formation of two separate layers. change in color was observed.
(g) Test for proteins: biuret test: determines the presence of peptide bonds in protein content in isolated biomaterial. in a test tube, 2 ml of biomaterial solution (0.1 gm. biopolymer dissolved in 2 ml of distilled water) was taken. added 1 ml of sodium hydroxide solution (1%) and 1% of copper II sulphate solution to above biomaterial solution drop wise. allowed the mixture to stand for 5 mins and observed the color change.
(h) Test for starch: added 2 drops of iodine solution to a test tube containing 2 ml of biopolymer solution (0.1 gm. biopolymer dissolved in 2 ml of distilled water) observed the change in color.
(i) Test for reducing sugar: Incorporated 1mL of Fehling's A and 1mL of Fehling's B solutions to a test tube containing 2 mL of biopolymer solution (0.1 gm. Biopolymer dissolved in 2 mL of distilled water). Heated at 60°C for few mins and observed change in color.

SPECTRAL STUDIES OF ISOLATED BIOPOLYMER:

IR Spectroscopy by KBr Disc Method. 1mg of isolated biopolymer was incorporated with 100 mg of Potassium Bromide in mortar to form pellet by applying pressure of 10 tons. Recorded IR Spectra. Similarly recorded IR spectra of pure Topiramate.

DSC (DIFFERENTIAL SCANNING CALORIMETRY):

Heat flow: 50-250°C, rate: 10°C/min and Nitrogen flow rate: of 20 mL/mins. DSC Spectra of biopolymer as well as that of pure Topiramate was recorded.

NMR (NUCLEAR MAGNETIC RESONANCE) SPECTRAL ANALYSIS:

Solvent: Dimethyl Sulfoxide), flow cell: 5 mm diameter. High flow rates were applied to sample. The flow cell in the instrument was rinsed again with the reaction mixture when the valve switches back. Recorded the Spectra.

SEM FOR SURFACE MORPHOLOGY OF ISOLATED POLYMER:

The surface topology of the biopolymer was studied by a Scanning electron microscope (SEM). This instrument produces signals through which surface topography, composition, morphological examination of the surface, and internal structure of the sample can be determined. SEM is also used for the elemental analysis of the biopolymer to give details of elemental composition.

MUCOADHESIVE PROPERTY OF ISOLATED POLYMER:

The isolated biopolymer was subjected to mucoadhesive property, and mucoadhesive was determined with the shear stress method. Firstly isolated biopolymeric solution was prepared using distilled water as a solvent in various concentrations ranging from 1-5% w/v. Each solution was used for determining in-vitro bond breaking strength or force required to break the bond at several contact intervals (5, 10, 15, 20, 25, and 30 min). A similar procedure was followed for standard polymers Sodium CMC and HPMC.

MUCORETENTIVE PROPERTY OF ISOLATED POLYMER:

Mucoretaintability was determined by M.S. Mucoretaintability method using an animal model of goat mucosa, i.e., Capra aegagrus labium as mucosal substrate using a thin film of biopolymer with phosphate buffer pH 6.5. The dislodgement time of bio-flexy film from the mucosal substrate was reported at fixed time intervals, and obtained data was compared with a standard film of Sodium CMC and HPMC polymer.

STANDARD GRAPH OF DRUG:

Preparation of standard curve of topiramate in distilled water:

Topiramate does not contain intrinsic chromophore, thus it cannot be analysed by ultraviolet, visible or fluo- rescence absorption without pre-treatment. A method was developed for Topiramate by the reacting it with Ammonium Molybdate as chromogenic agent in presence of 2M Hydrochloric Acid. 1, 2,3,4,5 mL of Standard drug solutions (10-50 µg/mL drug solution) was transferred in five 10 mL volumetric flasks. Added 2 ml of 5% of Ammonium Molybdate followed by 2 mL of 2M hydrochloric acid to above solutions. Made up the volume up to 10 mL with Distilled Water. Heated the reaction mixture in water bath for 35 mins at 50°C until full blue colour was developed. Measured the absorbance against blank.

PREPARATION OF STANDARD GRAPH OF TOPIRAMATE IN PHOSPHATE BUFFER OF PH 7.4

Dissolved 10 mg of Topiramate in 30 mL of Phosphate Buffer (pH 7.4) taken in a 100 mL volumetric flask. Made up the volume up to the mark with Phosphate Buffer (100 µg/ mL). Prepared dilutions of Concentrations (1,2,3,4,5,8,10,20,30,40,50 µg/mL) in 10 mL volumetric flasks. Absorbance was measured at λmax = 244 nm against solvent blank.

DRUG BIOPOLYMER INTERACTION STUDIES:

Topiramate : Isolated phoenix dactylifera biopolymer in ratios of 1:1, 1:3 and 3:1 were taken. Measured Absorbance and compared with pure Topiramate.

Dry method: Topiramate: phoenix dactylifera biomaterial in above mentioned ratios were taken in dry form in three petridishes. Kept for two hours at room temperature. Diluted the mixtures with 2 mL of Methanol. Absorbance was measured, observed shift in λmax in comparison with pure drug and reported.

Wet method: Topiramate: phoenix dactylifera biomaterial in above mentioned ratios were taken in dry form in three petridishes 1 mL of distilled water was added in each petridish. Dried in oven for 30 mins at 50°C. Diluted with 2 mL of Methanol. Absorbance was measured,

observed shift in λmax in comparison with pure drug and reported.

Colorimetry Method: Topiramate: phoenix dactylifera in ratio of 1:1 were mixed with Potassium Permanganate on glass plate. Observed color change, diluted suitably with distilled water, analyzed by UV. Repeated with Drug: Distilled Water and Drug: Potassium Permanganate.

NANOSIZING OF DRUG:

Solvent Evaporation Method: Admixed 100 mg Topiramate with, 10 mg of Dextrose, 5 mg of Fructose and 10 mL of Methanol in mortar pestle. Sonicated mixture for up to 5 cycles (each cycle of 180 secs). Diluted with 50 mL distilled water and further sonicated up to 15 cycles. Absorbance, % Transmittance, % Blockage (100 – % Transmittance) was measured after every 5 cycles. Dried the residue.

Sonication method: Admixed 100 mg Topiramate with, 10 mg of Dextrose, 5 mg of Fructose and 10 mL of Distilled Water in mortar pestle. Sonicated mixture for up to 5 cycles (each cycle of 180 secs). Diluted with 50 mL distilled water and further sonicated up to 15 cycles. Absorbance, % Transmittance, % Blockage (100– % Transmittance) was measured after every 5 cycles. Dried the residue.

SOLVENT CASTING METHOD AS FORMULATION TECHNIQUE OF BIO-FLEXY FILMS

Nanosized Topiramate (Anticonvulsant) (100 mg) was triturated with 50 mg of biopolymer (Mucoadhesive, film forming cum retarding agent) (in ratio of 1:0.5) for 2 min using pestle mortar. Added 10 m of Distilled Water (Solvent). To this dispersion, incorporated 10 mg of Dextrose (Flexicizer), 5 mg of Fructose (Flexicizer) and 10 µL of Glycerine (1% solution v/v) (Plasticizer) with continuous stirring. 0.6 gm. of Pectin (Film Initiator) was added. Mixture was further uniformly triturated for 5 min. Made up the volume up to 20 mL using Distilled water. Subjected the mixture to magnetic stirring for 15 min. Sonicated up to 5 cycles (each cycle 3 min). Clear dispersion obtained was poured into petridish. Kept for drying at room temperature for 24 hr. Removed prepared nanosized drug loaded Bio-flexy film from petridish. Similarly, six

different formulations of nanosized Topiramate with different isolated biopolymers and Standard Sodium Carboxyl Methyl Cellulose Polymer in different ratios of 1:1, 1:3, 1:5, 1:6 and 1:10 were prepared. (Tables 1, 2)

Table 1: Formulation of Nanosized Topiramate loaded Bio-Flexy Films using Glycine max Biopolymer.						
FORMULATION	FG01 (1:0.5)	FG02 (1:1)	FG03 (1:3)	FG04 (1:5)	FG05 (1:6)	FG06 (1:10)
Nanosized topiramate(mg)	100	100	100	100	100	100
Phoenix dactylifera biopolymer(mg)	50	100	300	500	600	1000
Dextrose(mg)	10	10	10	10	10	10
Fructose(mg)	5	5	5	5	5	5
Glycerine(µl)	10	10	10	10	10	10
Pectin(mg)	0.6	0.6	0.6	0.6	0.6	0.6
Distilled water	20	20	20	20	20	20

Table 2: Formulation of Nanosized Topiramate loaded Bio-Flexy Films using Sodium Carboxyl Methyl Cellulose Standard Polymer						
FORMULATION	FG01 (1:0.5)	FG02 (1:1)	FG03 (1:3)	FG04 (1:5)	FG05 (1:6)	FG06 (1:10)
Nanosized topiramate(mg)	100	100	100	100	100	100
Sodium Carboxyl Methyl Cellulose standard polymer (SCMC) (mg)	50	100	300	500	600	1000
Dextrose(mg)	10	10	10	10	10	10
Fructose(mg)	5	5	5	5	5	5
Glycerine(µl)	10	10	10	10	10	10
Pectin(mg)	0.6	0.6	0.6	0.6	0.6	0.6
Distilled water	20	20	20	20	20	20

Surface pH study: The formulations were immersed in 1 ml of distilled water for 1 hrs at room temperature. Measured pH using pH meter in triplicate and reported the avg. values.

Physical Appearance: The prepared bio-flexy films were checked visually according to the various parameters like texture, clarity, flexibility and smoothness in order to check the uniformity of prepared bio-flexy films. All prepared biofilms of phoenix dactylifera were found to be translucent, flexible, and smooth surface.

Tensile Strength: Tensile strength of the prepared bio-flexy films was determined by universal strength testing apparatus 17. The prepared bio-flexy films of a specific size (1 sq.cm) were fixed between glass plates, and strings and weights are applied until the bio-flexy films breaks. The tensile strength of prepared bio-flexyfilm was directly measured from weight required and reported. The tensile strength of prepared bio-flexy films loaded with topiramate was found in range of 91.32±0.99 g to 142.44±0.21 g.

Folding Endurance: The Folding endurance of formulated bio-flexy films was determined by continually folding the biofilm at the same place until it was broken. The number of times the bio flexy film could be turned at the specific position without cracking was recorded. The Phoenix dactylifera loaded bio-flexy films showed folding endurance from the range of 132.00±1.73 to 183.00±1.00 times.

Drug Content Uniformity: The bio-flexy films of 1sq. cm size from each prepared formulation was randomly selected and transferred into a volumetric flask (100ml) which containing 7 ml phosphate buffer (pH 6.5) and 1 ml methanol. The volumetric flask was stirred for 4 h on a magnetic stirrer. The achieved solutions were filtered through a 0.45µm membrane. The drug content was then determined after proper dilution using U.V Spectrophotometry through Shimadzu 1800 UV-Visible spectrophotometer 24 The drug content found in the bio-flexyfilm varied from 90.63±0.52% to 97.28±0.08%. The results of drug content uniformity showed that the topiramate drug was uniformly dispersed in all the bio-flexy films.

Stability Studies: The stability studies of the formulated bio-flexy films were determined for three months as per ICH guidelines at different temperatures and relative humidity. The formulated bio-flexy films were kept for stability studies in stability chamber at various conditions of temperatures and Relative Humidity (5 oC ± 3 oC /60% RH, at room temperature i.e., 25 oC ± 2 oC /60% ±5% RH and at 40 oC ± 2 oC / 75% RH) for three months. The changes were observed in the characteristics of bio-flexyfilms, and the results were reported.

CONCLUSION

The work done for the formulation of topiramate loaded in biopolymer (phoenix dactylifera) formulated into a bio-flexy films showed in-built film forming ability with appreciable mucoadhesivity. The future scope of labial delivery system is very a novel approach to develop various dosage forms as mucoadhesive tablet, mucoadhesive patches and mucoadhesive films, mucoadhesive etc.

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