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Research article

Formulation and evaluation of venlafaxine-loaded bio-flexi film for brain specificity via oro-trans soft palatal route

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ABSTRACT

The purpose of the present study was to formulate and evaluate venlafaxine loaded bio-flexi film for effective treatment of depression. For the preparation of bio-flexi film biomaterial was isolated from fruits of *Luffa acutangula* (L.) Roxb. (Cucurbitaceae) by an economic method. The biomaterial recovered from the concentrate was subjected for various physicochemical properties like color, solubility, color changing point and chemical test. Bio-flexi films were prepared by modified solvent evaporation method in different batches with variable drug/biomaterial ratio. Prepared batches were subjected for various evaluation studies like thickness, folding endurance, in vitro and in vivo drug release. Bio-flexi films were capable of releasing the drug in a slow sustained manner. From the present investigation, it may be concluded that biomaterial isolated from fruits of *L. acutangula* used in the preparation of bio-flexi film exhibit promising inbuilt mucoadhesivity for delivering venlafaxine at a controlled rate. So it can serve as a powerful natural mucoadhesant. It may significantly improve the ability to cross blood-brain barrier and serve as an effective tool to treat Depression disease.

INTRODUCTION

Depression is one of the most common psychiatric disorders with a life time prevalence of 10-20% in the general population and women being at twice the risk of developing depression compared to men (Rang et al., 2003). Literature suggests that prevalence of depression is higher in patients with chronic illnesses (Katon, 2003). Most of the drugs intended for treatment of depression enhance the availability of monoamines at the synapse by inhibiting their neuronal uptake or inhibiting their intraneuronal metabolism or increasing their release by blocking the alpha-2 auto and hetero-receptors on the monoaminergic neuron (Goswami et al., 2016). However, the efficacy of anti-depressant is often limited by their potential to reach the brain i.e., site of therapeutic action, blood brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier restrict the transport of drugs from systemic circulation into the central nervous system (CNS). It is estimated that more than 98% of the small new molecules do not cross the BBB, and hence fail to achieve the therapeutic concentration within the brain parenchyma cells (Goswami et al., 2016). Many approaches have been developed to circumvent this problem using liposomes, magnetic nanoparticles, solid lipid nanoparticles and polymeric nanoparticles (Zara et al., 2002; Shuting et al., 2010). The application of nano-technology for the drug delivery to the brain opens the doors of opportunities for the formulation scientists for the better and selective brain delivery of existing and newer potential molecules with CNS activity. It has been assumed that the delivery to brain of majority of the potential CNS drugs which have inability to cross BBB could be modified by Nano-technology in order to achieve better therapeutic action and better patient compliance (Goswami et al., 2016). Venlafaxine potentiate serotonin and norepinephrine in the central nervous system, through inhibition of their reuptake.

Luffa acutangula (L.) Roxb. (Cucurbitaceae), commonly called as Ridgegourd, is a popular vegetable in India known for its nutritional properties due to the presence of good amount of fiber, vitamins and minerals including vitamin B2, vitamin C, carotene, niacin, calcium, phosphorus, iron and small quantities of iodine and fluorine. It is reported to contain many phytochemicals such as flavonoids, saponins, luffangulin, sapogenin, oleanolic acid and cucurbitacin B. This plant has been extensively used in Indian traditional system

of medicines as diuretic, expectorant, laxative, purgative, hypoglycemic agent and a bitter tonic. The botanical survey revealed its use to protect from jaundice, insect bites, swollen hemorrhoids, dysentery and headache. Various biological activities of this plant were reported including its use in weight loss, jaundice, blood purification, hypoglycemia, constipation, skin care, immune system booster, wound healing, eye problems, stomach worms and asthma (Pullaiah et al., 2006).

MATERIALS AND METHODS

General

Venlafaxine was obtained as a gift sample from Ranbaxy, Ponta Sahib, Himachal Pradesh. The fresh fruits of *Luffa acutangula* were obtained from the local market of Dehradun, India. All other chemicals used were of analytical grade.

Isolation and characterization of biopolymer

The fruit pulp of *L. acutangula* was separated; skin was removed and grinded in mixer grinder with distilled water and filtered. The filtrate was centrifuged at 4000 rpm for 5 min and the supernatant was then treated with acetone and set in refrigerator for overnight. The biomaterial was collected by centrifugation and dried at room temperature. After that the biomaterial was passed through sieve 120. The isolated biomaterial was subjected for various physicochemical tests like colour, odour, solubility, colour changing point, etc.

Preparation of bio-flexi film

Accurately 100 mg of *L. acutangula* biopolymer was weighed and transferred into the mortar; to this, 110 mg of dextrose was added and triturate the mixture for a period of five minutes. After that, 5 mg of venlafaxine was added in geometrical dilution pattern. Further, 10 ml of double distilled water was added drop by drop to the mixture with constant trituration. The mixture was subjected for magnetic stirring for a period of 10 min and sonicated at 400 Hz for 3 cycles of 1 min each in order to form a colloidal mixture. The colloidal mixture was poured into petridish of 6 cm diameter and subjected for evaporation at room temperature for a period of 10 h. Dried films were carefully removed. Similarly, six formulations (F1-F6) were prepared by varying the concentrations of *L. acutangula* polymer (Table 1). Two standard formulations (L7 and L8) were also prepared using sodium alginate and sodium carboxy methyl cellulose.

Table 1. Formulations of *Luffa acutangula* polymer, sodium alginate and sodium carboxy methyl cellulose.

S.No.	Ingredients	F1	F2	F3	F4	F5	F6	L7	L8
1.	Venlafaxine (mg)	5	5	5	5	5	5	5	5
2.	*L. acutangula* (mg)	100 (1%)	200 (2%)	300 (3%)	400 (4%)	500 (5%)	600 (6%)	-	-
3.	Sodium carboxy methyl cellulose (mg)	-	-	-	-	-	-	400 (4%)	-
4.	Sodium alginate (mg)	-	-	-	-	-	-	-	400 (4%)

Characterization of biopolymer

Physical characterization

The extracted biopolymer was evaluated for physical characteristics like appearance, odour, solubility, percentage yield, swelling ratio, pH value, charring, and density as per method earlier method by Madhav and Yadav (2013).

Chemical characterization

The extracted biopolymer was tested for chemical characteristics (Ojha, 2012) like test for carbohydrates, test for chlorides, and test for sulphate.

FTIR spectroscopy

The dried biopolymer was subjected to FTIR spectroscopy using FTIR 1601 (Shimadzu, Tokyo, Japan). The samples were prepared by solid sampling technique using potassium bromide pellets. The scanning range was fixed at 400-4000 cm^{-1}.

Characterization of bio-lip strips

Thickness

The thickness of three randomly selected bio-flexi films was assessed at five different places (centre and four corners) on a single patch of each formulation using a micrometre screw gauge and the mean value was calculated and reported (Madhav and Ojha, 2012).

Weight uniformity study

Weight uniform study for all formulated bio-flexi films was performed by taken three randomly selected bio-flexi films from each formulation with surface area 1 cm^2. Each film was weighed individually on electronic balance. The

study was performed thrice and average weight was calculated.

Content uniformity

All formulated bio-flexi films were evaluated for their drug content uniformity. From each formulation, the randomly selected film (1 cm^2) was transferred into a 100 ml volumetric flask containing 7 ml of phosphate buffer of pH 7.4 and 1 ml of methanol. The flask was stirred for 4 h on magnetic stirrer. A blank was prepared using a drug free patch treated similarly. The solutions were filtered through a 0.45 µM membrane (Madhav and Ojha, 2012). The drug content was then determined after proper dilutions using an UV spectrophotometer.

Folding endurance

Folding endurance for all bio-flexi films containing venlafaxine was performed by using a film of 4 cm^2 area from each formulation. The selected bio-flexi film was subjected to folding endurance by repeatedly folding a strip at the same place until it broke. The number of folding required to break or crack a strip was taken as the folding endurance. This test was repeated thrice and overcome was noted.

Swelling index

Swelling study of all formulated bio-flexi film was calculated by taken a bio-flexi film from each formulation of size 1 cm^2. The bio-flexi film was weighed on a pre-weighed cover slip. It was kept in a Petri dish and 10 ml of phosphate buffer of pH 7.4 was transferred. After one hour, the cover slip was removed and weighed. The difference in the weights gives the weight increase due to absorption of water and swelling of bio-film. The change in weight was noted after 24 hrs (Madhav and Ojha, 2012). The procedure was repeated thrice and swelling index(S) was determined by a formula as given below.

$$\%S = (X_t - X_o / X_o)$$

Where, Xt denoted for weight of the swollen bio-flexi film after time t and Xo for original weight of bio-flexi film.

Mucoadhesive Property

The mucoadhesive property of isolated material was evaluated by Shear Stress method. The biomaterial was subjected to a shear stress study for in vitro assessment of its adhesive strength in terms of weight required for breaking adhesive bonds between polymer and glass plate in a specified contact time of 5, 10, 15, 20, 25 and 30 min with concentrations ranging from 1% to 5% w/v. It was compared with the standard polymer sodium carboxy methyl cellulose and sodium alginate (Ojha and Madhav, 2012).

RESULT AND DISCUSSION

Physical characterization

The extracted biopolymer was brown in colour and soluble in water and methanol. The pulp gave 25.5±1.1 g of yield per kg and the swelling index was found to be 42±3.5%. The results are shown in Table 2.

Table 2. Physical characterisation of *Luffa acutangula* biopolymer.

Physical properties	Observation
Appearance	Brownish powder
Odour	Characteristic
Solubility	Water, methanol
Yield (g/kg)	25.5±1.1
pH	7.0±0.4
Swelling index (%)	42±3.5

Chemical characterization

The biopolymer obtained from *L. acutangula* gave positive test for carbohydrates and negative tests for alkaloids, protein, chlorides and sulphates. The results based on preliminary phytochemical analysis are given in Table 3.

Table 3. Chemical characterization of *Luffa acutangula* biopolymer.

Chemical Properties	Observation
Molish test (for carbohydrates)	+
Ninhydrin test (for protein)	-
Wagner's test (for alkaloid)	-
Silver nitrate test (for chlorides)	-
Barium chloride test (for sulphates)	-

Symbol: (+) = present; (-) = absent.

FTIR spectrum

The FTIR spectrum of biopolymer obtained from *L. acutangula* showed characteristic peaks at 2934 (C-H stretching), 2363 (O-H stretching), 1731 (C=O stretching) and 1419 cm^{-1} (C-O stretching). The FTIR spectrum of biopolymer obtained from *L. acutangula* is shown in Fig. 1.

Evaluation parameters of venlafaxine loaded bio-flexi films

The formulated films were smooth and translucent in appearance. The average thickness of all prepared bio-flexi films ranged from 0.45±0.03 to 0.73±0.07 mm. Thus the proportional gain in weight of films was observed as the thickness of films increased. The values were uniform for the films within the respective group of formulation type. This depicts that the films cast

was uniform. Surface pH for all formulations was found to range from 6.34±0.3 to 6.83±0.2. Since range of the pH of film is near to the oral mucosal pH, no irritation was expected. The folding endurance of films was found in the range of 145±4.8 to 188±5.6. High folding endurance values for films indicates high mechanical strength of films. This is highly desirable because it would not allow easy dislocation of the films from the site of application or breaking of film during administration. The results based on venlafaxine-loaded bio-flexi films study are given in Table 4.

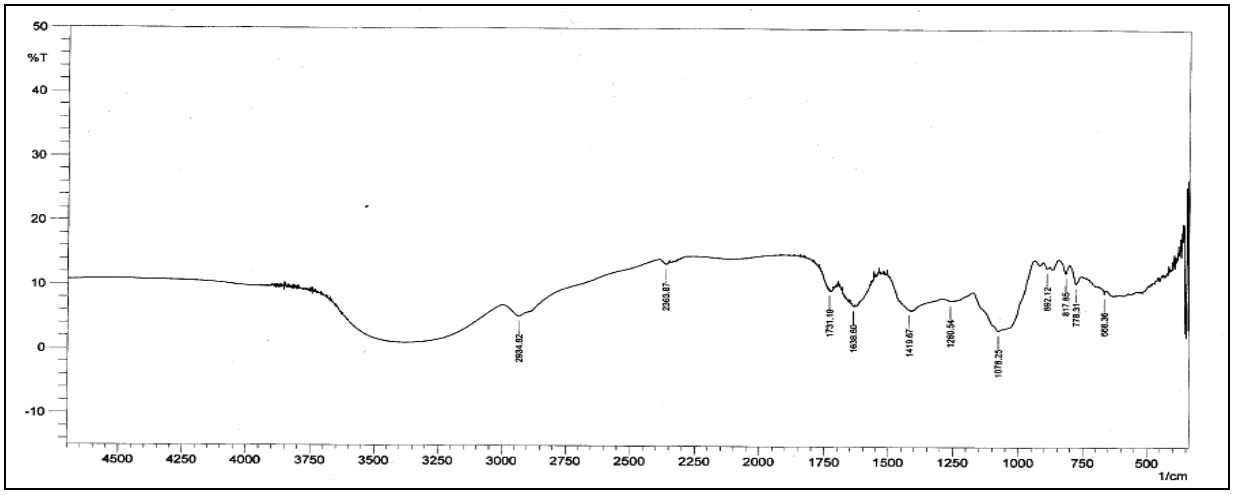

Fig. 1. FTIR spectrum of biopolymer obtained from *Luffa acutangula.*

Table 4. Results of evaluation of various batches of venlafaxine-loaded bio-flexi films.

Formulation	Content uniformity (%)	Weight uniformity (mg)	Swelling index
F1	95.25±0.59	23.29±0.21	148.58±0.32
F2	96.32±0.41	26.13±0.27	117.48±0.53
F3	93.15±0.45	28.34±0.25	136.76±0.63
F4	95.73±0.52	22.75±0.35	123.37±0.66
F5	89.81±0.50	21.82±0.37	142.44±0.68
F6	96.42±0.48	27.35±0.31	109.76±0.71
F7	95.25±0.59	23.29±0.21	148.58±0.32
F8	96.32±0.41	26.13±0.27	117.4±0.53

In vitro release

In vitro release of venlafaxine from different films is shown in Fig. 2. Formulations F1 to F8 showed drug release in a controlled manner. Formulation F3 showed the maximum release of 90.06% at the end of 24 h. The results showed that initially drug release decreases with increasing the concentration of biomaterial and further drug release increased. We could not detect any exact relationship between the drug release profile and polymer composition may be due to release mechanism which governed by diffusion as well as erosion controlled, since our biomaterial is slightly soluble in water. The release data of the tested strips were analysed on the basis of Korsmeyer-Peppas equation and Higuchi kinetics (by BIT-SOFT 1.12: drug release kinetics with model fitting). Coefficients of correlation (R^2) were used to evaluate the accuracy of fit. The R^2 value for Higuchi and Peppas kinetic models were calculated and compared. All the tested formulations gave good fit to the Korsmeyer-Peppas model (Fig. 3). All formulations showed non-Fickian drug release ($0.5<n<1$). The in vitro release obtained by bio-flexi films were significantly ($p<0.05$) different from standard formulations. On the basis of above parameters and used concentration of biomaterial F3 was selected as the best formulation. The in vitro studies have shown that this is a potential drug delivery system for venlafaxine with considerable good stability and release profile.

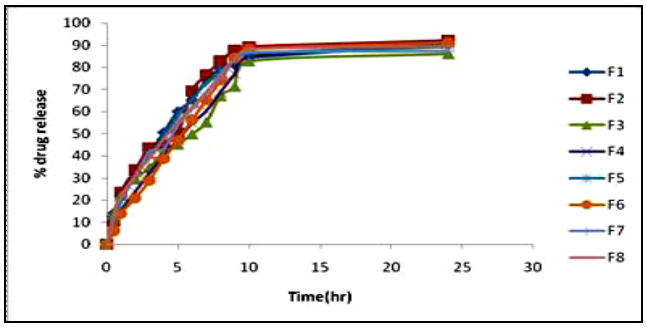

Fig. 2. In vitro drug release profile for batch F1-F8

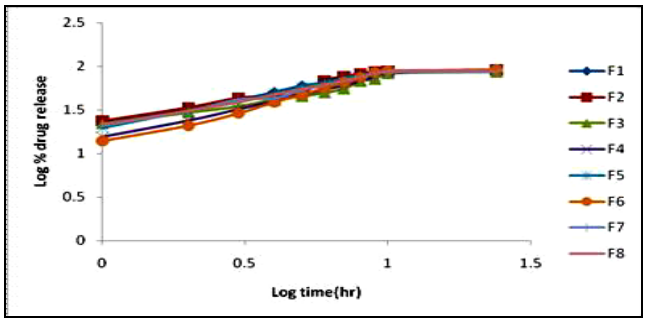

Fig. 3. Korsmeyer-Peppas model for venlafaxine-loaded bio-flexi films

Evaluation of mucoadhesive property

The shear stress study revealed that 5% concentration showed promising mucoadhesivity as compared to that of standard sodium carboxy methyl cellulose and sodium alginate polymer. The results related to the mucoadhesive properties are depicted in Table 5 and Fig. 4.

Table 5. Mucoadhesivity of bio-films and standards

Sample	Mucoadhesivity at different contact time					
	5 min	10 min	15 min	20 min	25 min	30 min
Water	7.0	11.5	13.6	16.6	19.5	22.0
LA (1%)	8.3	10.5	15.6	17.4	20.0	30.3
LA (2%)	14.7	20.6	26.0	30.7	39.6	45.4
LA (3%)	20.3	26.5	34.0	37.6	39.8	43.6
LA (4%)	29.8	34.7	39.9	49.8	79.7	92.3
LA (5%)	50.4	59.4	70.2	93.9	118.3	164.3
SCMC (3%)	59.6	58.3	72.2	97.3	115.3	160.5
SA (3%)	49.8	56.3	76.3	90.3	120.7	170.3

Abbreviation: LA (*Luffa acutangula*); SCMC (Sodium carboxy methyl cellulose); SA (Sodium alginate).

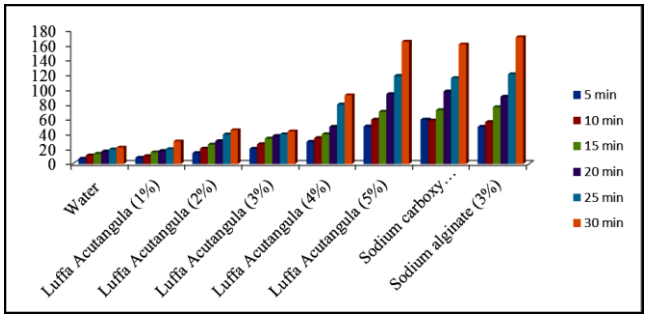

Fig. 4. Mucoadhesive property determined by shear stress method

Conclusion

In the present study, bioadhesive bio-flexi films based on *L. acutangula* biomaterial was developed. Thus, this natural biomaterial could be promising excipient for drug delivery. Also, the biomaterial has promising inbuilt mucoadhesive property. It can be used an alternative to conventional synthetic or semisynthetic mucoadhesive agent.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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