Original Article

Formulation And İn Vitro Evaluation Of Site-Specific Local Action Dental Films Of Doxycycline Hyclate For Treatment Of Periodontitis

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Abstract

The work was aimed to formulate intrapocket dental films with rate controlling polymers, capable of delivering therapeutic concentrations of doxycycline hyclate (DCH)for a prolonged period and study the antibacterial activity for local action. Intrapocket dental films of DCH were formulated by solvent casting technique using HPMC E15, eudragit RS 100, PEG 400, dibutyl phthalate. The dental films were optimized on basis of physicochemical parameters such as FTIR, DSC, thickness, uniformity of weight, drug content, surface pH, percentage moisture loss, percentage moisture absorption, tensile strength, swelling index, folding endurance, in vitro bioadhesion test, in vitro drug release and in vitro antibacterial activity. FTIR and DSC showed compatibility between the drug and excipients. The formulated films were flexible, smooth, homogenous, non-sticky and revealed acceptable mechanical and physicochemical characteristics (F11 and F12). The films showed sustained in vitro release for 7 d. Kinetic models indicated DCHrelease from these dental films was best fit towards first-order kinetics. It is concluded that local therapy of periodontitis with the help of DCHloaded HPMC E15 and eudragit RS 100 dental films have a remarkable role. This combination of HPMC E15 and eudragit RS 100 can be used to develop dental films for other drugs.

Keywords: Periodontitis, dental film, doxycycline hyclate,bioadhesion,tensile strength, antibacterial activity.

INTRODUCTİON

In industrialized countries 60-90% of school children and a majority of adults are affected by dental-related problems(Nazir, 2017). According to the World Health Organization (WHO), developing countries have higher prevalence of calculus and bleeding among adolescents (WHO, 2020).Periodontal disease is a chronic oral diseases which causes tooth loss and threatens oral health (Hoare et al., 2019). More than 50 % of the Indian community across all groups, ethnicities, races, genders and socioeconomics levels get affected by periodontitis (Peres et al., 2019; Kassebaumet al., 2017).While antibiotics are administered to manage these conditions, the conventional systemic antibiotic products fail to achieve the desirable effectiveness because of inadequate availability at the target site. Consequently, use of higher dose and prolonged administration of systemic antibiotic product leads to adverse effects including gastrointestinal disorders and the development of antibiotic resistance (Himansuet al., 2017; Yanget al., 2018).Besides these non-compliance, it also increases the cost of the

treatment(Samalet al., 2015). Accordingly efforts have been made for local delivery of antibitiotics to achive higher availability at the periodontal site of infection.This is based on the rationale that the locally delivered antibiotic can easily reach the site of action and get localised for adequate duration to achieve the desirable therapeutic concentration. However, presence of bacteria in biofilm and rapid clerance of antibiotic by gingival crevicular fluid (GCF) may challenge the effectiveness.For example, short residence time at the site of application is the major disadvantage of the oral gel as it gets cleared bysaliva.So frequent application to maintain effective concentrations adversely affects patient compliance (Himansuet al., 2017; Rani and Singh, 2018). Therefore, developing the prolonged-release drug delivery system has recieved wide researchattention.

Dental films are the most widely used form of medicated intrapocket dental drug delivery device for prolonged release of the drug. These are safe and effective for low dose drug delivery devices and provide a flexibile, comfortable and acceptable approach for local drug delivery (Urmi et al., 2016). In film matrices drugs are uniformly distributed with polymers and release of drugs follows diffusion and/or erosion mechanism (Chen and Wang,2010). The release is influenced by several parameters including the concentration of the film-forming polymers, plasticizers, evaporating solvents and physiochemical properties. The dimensions and shape of the film can be controlled to match the application requirement and patient-compliance. With less storage space, good retention, less maintenance of machinery and minimal or no side effects, dental films can be considered suitable for achieving the desirable therapeutic effectiveness with better patient compliance.

The recent advances in the field of dentistry have promoted the use of various broad-spectrum antibiotics for treatment of periodontal diseases. DCHis popular among dentist for treatment of periodontitis (Ana and Hérida, 2012; Weiner and Buhimschi, 2009). Cross-resistance is common, although some Staphylococcus aureus resistant to tetracycline,are reported to respond to doxycycline(Emaneini et al., 2013). It has been proved that doxycycline is more effective than tetracyclines in manging these dental infections(Taner et al., 1994).

To achieve desirable therapeutics concentrations and effectively manage periodentitis several formulation strategies have been adopted fordoxycycline. These include film (Mahmoud and Samy, 2016),strip (Ozcanet al., 1994), gel (Guptaet al., 2008; Polsonet al., 1997), microparticle/microsphere (Rao et al., 2012; Aishwaryaet al., 2008; Mundargi et al., 2007), nanoparticle (Wayakanonet al., 2013),liposome(Jin et al., 2010)and nanofiber (Chaturvediet al., 2013). The biodegradable mixture of doxycycline gel and powder (in syringe) are available in the market as Atrigel and Atridox by Atrix labs, Ft., Collins (Joshiet al., 2016). However, frequent administration and flush-out during mouth washing limit the compatibility and efficacy of the gels. Further, strips and fibres are associated with issues including dislodging, pain during insertion and high drug loading. Thus there have been efforts to develop alternative films for doxycycline. Poly(vinyl alcohol), chitosan, poly(DL-lactide-co-glycolide, gelatin, ethyl cellulose, poly(DL lactic acid) have been used to develop dental films (Mahmoud and Samy, 2016; Joshiet al., 2016).In a recent study, resorbable material hydroxypropyl methylcellulose (HPMC)with carbopol 934were used to develop films for metronidazole (Labibet al., 2014). Encouraged by this we thought it worthwhile to use HPMC along with eudragit to develop dental films for doxycycline. Therefore, the present study was aimed to formulate and evaluate intrapocket periodontal dental films of DCHwith rate controlling polymers for prolonged action and antibacterial effects.

MATERIALS AND METHODS

Materials

DCHwas obtained as a gift sample from Meenaxy Pharma Pvt Ltd. Hyderabad, India. Ethylcellulose and PEG 400 was obtained from Otto Kemi, Mumbai. Eudragit RS 100 and HPMC E15 were purchased from Yarrow chem. Products, Mumbai, India. Chloroform, dichloromethane and dibutyl phthalate were from Thermo Fisher Scientific, India. Lotus enterprises, Andhra Pradesh, India supplied the remaining required analytical grade of chemicals and reagents.

Table 1: Formulation design of DCH dental films

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
DCH (mg)	-	-	100	120	120	120	150	100	120	150	175	200

Ethyl cellulose (mg)	750	1000	1000	880	1000	800	800	-	-	-	-	-
Eudragit RS-100 (mg)	250	200	200	200	-	200	-	200	200	200	200	150
HPMC K4M (mg)	-	-	-	-	100	-	-	-	-	-	-	-
PVP K30 (mg)	-	-	-	-	-	-	200	-	-	-	-	-
HPMC E15 (mg)	-	-	-	-	-	-	-	650	750	800	800	850
PEG 400 (ml)	1	0.1	0.1	0.1	0.1	1	1	0.2	0.2	0.2	0.1	0.3
Dibutyl phthalate (ml)	-	0.3	0.3	0.3	1	1	1	0.3	0.3	0.3	0.1	0.3
Ethanol (ml)	10	-	-	-	-	10	20	-	-	-	-	5
Chloroform: Dichloromethane (ml)	-	1:1	1:1	1:1	1:1	1:1	-	1:1	1:1	1:1	1:1	1:1

Preparation of dental films

Dental films were developed by solvent casting techniques. HPMC E15 and Eudragit RS 100 were dissolved alone and also in combination of 10 ml ethanol. Chloroform and dichloromethane were added to dissolve completely using magnetic stirrer (1-MLH, Elektrotechnik Ltd., India) to get the different concentration of polymer solution. The required quantity of DCHand plasticizer (dibutyl phthalate and PEG 400) were added to the polymeric solution with continuous stirring assuring complete mixing. Cleaned petri dish was placed on a horizontal plane and the solution was poured in it. The cotton plugged glass funnel was placed (inverted position) over the petri dish that allowed the solvent to evaporate slowly at 25 ºC for 24 h. The prepared dental films were kept at room temperature for 2 d followed by hot air oven (1 h at 30 ºC) (Model: KOMS.3, Kadavil Electro-Mechanical Industries, India) for complete evaporation of the solvent. Aluminum foil wrapped indivisual films were stored in desiccators (Karkiet al., 2016). The films were cut into required size (7 × 4 mm) with the help of sharp knife for further studies as the periodontal probing depth is around 4 to 7 mm.

Fourier- transform infrared spectroscopy (FTIR)

The prepared formulations, pure drug, polymers/excipients were scanned from 4000 to 400 cm^{-1}at a constant resolution of 2 cm^{-1} using FTIR (Cary 60, Agilent Technologies, USA). The FTIR spectra were obtained for characterization of functional groups. FTIR studies help in studying, whether there are major interactions between the drug and the polymer/excipient that may lead to the formation of new bonds.

Differential scanning calorimetry (DSC)

The thermal properties of DCH(pure drug), polymersand developed optimized film (F12)was performed. Each 3 mg of samples were enveloped in sealed aluminum pans. The samples were heatedover a temperature range of 30-250 ºC at a constant rate of 10 ºC/min, under nitrogen as purge gas (150 ml/min) using PerkinElmer, Pyris Diamond (Singapore).

Thickness

Assessment of thickness was done using digital micrometer (0-25 mm, Wenzhou Sanhe measuring instruments co. Ltd., China) at ten different positions (corners and center).

Uniformity of weight

Ten strips were cut from different places of the same formulation film and indivisual weight was determined using digital balance (BL-220H, Shimadzu Corporation, Japan). The S.D. of weight variation was compared with the mean value.

Drug content uniformity

Films (7 × 4 mm) were placed in 10 ml of simulated gingival fluid (pH 6.8) in a volumetric flask and stirred under magnetic stirrer at 25 ºC for 24 h and then subjected for ultrasonication (15 min). The solution was filtered through membrane filter paper(0.45 µm) and quantified by UV Visible spectrophotometer (Model: cary 60, Agilent technologies, USA) after doing appropriate dilution (Ali et al., 2012).

Folding endurance

Folding property was evalautedfor desired texture of the films. Folding endurance was evaluated by folding (180° angle) the developed film at the same place repeatedly until it breaks (Mayet et al., 2014).

Surface pH

Warmed agar solution (2 % w/v) was poured into petri dish for gelling at room temprature. Periodontal developed films were placedon the agar gel and allowed to swell for 1 h. The pH paper was placed on the suface of the swollen films to determine the suface pH (Ahujaet al., 2006).

Tensile strength and percentage of elongation

The periodontal developed films were evaluated thrice for each batch for the percentage of elongation and tensile strength (mechanical properties) using an Instron Universal testing machine (Model 2046, Instron Ltd, Japan).The film was fitted between two clamps at a distance of 1cm and pulled by a top clamp at a rate of 5mm/minutes. The film broke into two pieces are the indicators for the determination of the breaking force and elongation(Chauhanet al., 2018).

In vitro bioadhesion test

In-vitro bioadhesion testingof the developed films of DCHwas evaluated using texture analyzer(TA.XT Plus, Stable Micro System, UK). This method was adopted from reported method with slight modification(Chauhanet al., 2018). Goat excised mucosa membrane was collected from the local slaughterhouse was used for the study. Maximum detachment force (F_{max})required to separate probe from mucosa was determinedusing the Texture Exponent 32 Software. Area under the force and distance curve gives the work of adhesion.

Percentage (%) of moisture loss and moisture absorption

Weighed cut films were placed in desiccator containing anhydrous calcium chloride at room temperature in a dark place. The strips were removed after 3 dand reweighed for the calculation of % moisture loss. Pre-weighed known sizes of dental films were placed in a desiccator containing of saturated solution of aluminium chloride (100 ml) and maintained 75% of relative humidity. The films were removed after 3 d and reweighed for the calculation of % moisture absorption.

Swelling index

The pre-weighed developed films (7×4 mm) were immersed in simulated gingival fluid. The films were removed from the fluid at definite time intervals. The excess fluids adhered to the films were removed by tissue paper.The films were reweighed immediately. The process was continued till constant weight of the swelled films and the swelling index was calculated.

In vitro antibacterial activity

Itwas performed using 20 ml of nutrient agar media (sterilized- pressurE15 lb, time 20 min) and 0.1 mL of Streptococcus aureussuspension (spreading method) (Kassebaumet al., 2017). The incubation condition was mentained at 37 ˚C for 7 d. The film strips (7 × 4 mm) were transferred onto freshly seeded agar plates for an additional 48 h for incubation. Then, the zone of inhibition was measured using “Hi Antibiotic Zone scale”.This procedure was repeated until no inhibition was obtained.

In vitro drug release

The films were remain immobile in the periodontal pockets. So, the dissolution of the developed dental films were performed in static dissolution method. Two ml of simulated gingival fluid were taken in glass vials and maintained at 37 ± 0.5 ºC. Sets of six films (triplicate) of known weight and dimensions were placed separately

into it. At predetermined time intervals (1, 4, 8, 12 h, 1, 2, 3, 4, 5, 6, 7 d), 1 ml of sample was withdrawn and replaced with a fresh preheated (37 ± 0.5 ^{o}C) buffer. The concentration of doxycycline was determined using UV visible spectrophotometer at 270 nm with suitable dilution (Kassebaumet al., 2017; Emaneini et al., 2013).

Kinetic analysis of in vitro release data

The results of in vitro release profiles obtained for the formulations were plotted in kinetic models of the data treatment (Peppaset al., 1985; Korsmeyeret al., 1983; Higuchiet al., 1963)

RESULTS AND DISCUSSION

Formulation design

Periodontal films were developed by industrial feasible solvent casting technique using DCHalong with co-polymers HPMC E15, eudragit RS 100 and plasticizers (PEG 400 and dibutyl phthalate). The chloroform, ethanol and dichloromethane were used as evaporating solvents. Twelve different formulations were designed with the combinations of drug, polymers, plasticizers and solvents with varying the concentrations (Table 1).The drug was not incorporated into the formulations F1 (Fig. 1A) and F2. The produced films were smooth, homogenous, non-sticky and flexible.As the concentration of the ethylcellulose was increased (F2) the nature of films becomes more flexible. The flexibility of the ethylcellulose depends upon the solvents andplasticizers used. The plasticizers PEG 400, dibutyl phthalate, solvent mixture of chloroformand dichloromethane showed good flexibility and alsoit may control the release for prolonged period of time.Soft, sticky and non homogeneous films weredeveloped upon addition of drug into the formulation (F3).Increase in the concentration of drug and decrease in proportion of polymers affected homogeneity of the films.The film produced by F5 was found to be sticky and brittle may due to the lack of eudagit RS 100 and presence of HPMC K4M. The removal of HPMC K4M (F6) and inclusion of PVP K 30 (F7)were found to produce brittle films.In contrast to these the F8 formulation with HPMC E15 polymer in the given ratio formed flexible films.However, the films were not uniform throughout the surface. Increase in the concentration of HPMC while keeping other excipients and solvents constant adversely affected the formation of films. This may be due to the fact that the solvents including ethanol, chloroform and dichloromethanewere not completely evaporated at room temperature (Sadeghi et al., 2011).The F10 formulation produced flexible films which were not smooth whereas F11 formulation formed smooth, homogenous, non-sticky and flexible films (Fig. 1B). Eventhough the drug concentration was increased while keeping other excipients constant, it produced smooth, homogenous, non-sticky and flexible films in the formulation F12 (Fig. 1C). As plasticizers impact flexibility of the films by reducing its brittleness, combination of PEG 400 and dibutyl phthalate showed good plasticizing effect with desired film properties. The HPMC showed good film-forming properties. Further its acceptability is due to formation of transparent thin films with adequate flexibility and good mechanical strength. Besides these films can be expected to undergo rapid disintegration due to its water solubility while providing a good mouthfeel effect (Dinge and Nagarsenker, 2008).The formulationsF11, and F12 cut into required size (7 x 4 mm) as per the periodontal probing depth (Fig. 1D).It was not visible to the outside as it was transparent and flexible. These were help for easy insertion and fixing between the tooth or periodontal pocket for release of drugs.

FTIR

The FTIR spectrum of DCH, HPMC, eudragit, dibutyl phthalate, PEG 400 and optimized formulation (F12) are shown in Fig. 2. The DCHFTIR spectrum was similar to that of the earlier reported data by Kogawa et al., 2014. It showed peak at 3278.2 cm^{-1} due to the N-H stretching vibration. The peaks observed at 1891.6 and 1656.8 cm^{-1} were assigned to the C=O streching of the carbonyl group and the amide group respectively.The characteristic peaks for eudragit were observed at 1653.1 (C=O) and 1146.2 cm^{-1} (C-O). HPMC showed characteristic bands at 3451.5 (O-H), 2901.7 (C-H), and 1053.1 cm^{-1} (C-O). PEG (polyethylene glycol) showed the characteristic bands at 3322.9 (O-H-strecching) and 1038.1 cm^{-1}(C-O-streching). Dibutyl phthalate showed characteristic bands at 1725.8 (C=O-strecching) and 2961.4 cm^{-1} (C-H-strecching). FTIR spectra of optimized formulation (F12) showed characteristic individual peaks without any major shift. This indicates that there are no newly formed bonds and the structural integrity of the DCHis maintained in the formulation. Thus, there is acceptable compatibility and the excipients can be used safely to formulate dental films.

DSC

DSC thermogram of pure drug DCH, polymers (eudragit and HPMC) and optimized formulation (F12) are displayed in Fig. 3. The freeDCHexhibited a single sharp endothermic melting peak at 181.69 ºC (ΔH = 94.50 J/g). The melting peak of HPMC was at 93.64 ºC whereas eudragit did not show any endotheric peak.Absence of anynew peaks indicates the compatibility between the DCHand excipients. The optimized formulation showed broad endothermic peak corresponding to the drug (Fig. 3D). This indicates the transition from crystallinity to amorphous forms which may be due to the homogenous mixing of the drug and polymers at molecular level.This may enhance the drug solubility and help in the development of dental films.

Thickness uniformity of the films

Optimisation of the thickness of the film is important for easy insertion to the pocket and avoid discomfort. A thickness of less than 0.4 mm and adequate adhesiveness are desirable to preventdislodging during routine mouth washing (Labibet al., 2014). The thickness of the formulations F11 and F12 was found to be 0.31 ± 0.01 mm and 0.35 ± 0.02 mm respectively (Table 2). Thus the dental films haveappropriate an uniform thickness to enable higher contact time.

Uniformity of weight of the film

To support the thickness unformity, the weight uniformity was assessed. The drug loaded dental films were cut into 7 × 4 mm size (Fig. 1D) and weighed. The average weight of the dental films of the F11 and F12 were 0.012 ± 0.001 and 0.013 ± 0.001 mg respectively. The reproduciblity in weight of the fiims indicates the weight uniformity. Thus the unifirmity in weight and thickness suggest the drug content uniformity of the formulations.

Drug content

To validate the drug uniformity as suggested by uniform thickness and weight of the formulations, the content of drug was assayed. The drug content of the developed formulations F11 and F12 were found to be 88.33 ± 2.85 % and 86.45 ± 1.55 % respectively (Table 2). This indicated uniformity in drug content. It represents the equal distribution of DCH in all developed dental films of the same batch. This suggests that the films were reproducible and the method as well materials used in the preparation are reliable(Ali et al., 2012).

Folding endurance

The folding endurance is estimated as the number of folding times. This is a measure of the flexibility that is neccessary forease of insertion/application of the filmin the periodontal pocket. A value of ≥ 100number of folding times is considered desirable for a dental film (El-Kamel et al., 2007). The folding endurance for the formulation F11 and F12was198 ± 3and 222 ± 3 respectivelly (Table 3). The better folding endurance of the formulation F12 can be due to the relatively higher level of plasticizers.While increase in plasticizer enhance flexibility, further increase in plasticizer may reduce the adhesiveness. Thus it is important to have balance of these properties in the films (Labibet al., 2014). Thus F11 and F12 with good adhesiveness and higher flexibility can beconsidered suitable for application as dental films.

Tensile strength and percentage of elongation

Mechanical properties are important for the films. It determines the ability of a film to withstand the rupture and force required to break. The formulation F12 showed the higher tensile strength (4.76 MPa) and the lower percentage of elongation (5.04 %) compared to the F11 formulation (3.84 Mpa, 7.86%).This is generally attributed to the degree of crosslinking between the polymers that produce films and confer higher rigidity to the film (Karki et al., 2016).

Surface pH

Both acidic and basic pH of the films can cause incompatibility issues including irritation at the site of application leading to patient discomfort. It may also lead to the dissolution of enamel and demineralization of teeth. To overcome these problems, the pH of the developed dental films must be closer to the pH of the gingival cervical fluid. Thus, the surface pH of the films was determined to investigate the local effects or compatibility with the oral cavity. The surface pH of the formulations F11 and F12 were found to be 6.52 and 6.82 respectively (Table

3). As this is close to the oral pH, no periodontal pocket irritation is expected for both the formulations (F11 and F12) (Ali et al., 2012).

In vitro bioadhesion test

Adhesion of the films in the periodontal pocket is critical to the control release of drug at the local site of application. Textural characteristics of the developed periodental films are not only for the ensuring the durability in the site of application but also for the patient compliance. Bioadhesion of the films depends upon the polymers chain, degree of hydration and swelling. Hydration helps in expanding polymer chain and exposing more available sites for bond formation. The inter-diffusion and entanglement of expanded polymer chains and formation of secondary bonds produce the bioadhesion. It can be enhanced by increasing contact time and applied force(Mayetet al., 2014).According to literature review, non-ionic polymer HPMC had moderate mucoadhesive property and eudragit displayed promising bioadhesive properties (Karki et al., 2016).Combination of HPMC and eudragit showed better bioadhesive property. The films prepared with HPMC and eudragit (F12) showed higher adhesiveness (0.21 ± 0.05) than the formulation F11 (0.17 ± 0.07)(Table 3). These results can be explained by the amount of HPMC, eudragit and plasticisers present in the formulations. Higher the amount of HMPC, plasticisers and lower eudragit amount in F12 formulation showed promising bioadhesive property than F11 formulation. In our study, combination of HPMC and eudragit in specified amountwas found to be more effective bioadhesive property of the developed films.

Percentage of moisture loss and moisture absorption

The amount of the moisture plays an important role in the film properties like adhesive, mechanical strength. Moisture loss studies were conducted for the formulation F11 and F12 and reported in Table 2. The percentage moisture loss of formulation F11and F12 was 0.03 ± 0.01 and 0.022 ± 0.01 respectively. The formulation F11 showed maximum percentage moisture loss as compared with F12 formulations. The amount of moisture loss was very less in both formulations. These results indicates stability of the formulation and retension of the films properties. The percentage moisture absorption of the formulations F11 and F12 were 0.04 ± 0.01and 0.09 ± 0.02respectively (Table 2). Formulation F12 showed the highest percentage of moisture absorption due to the increase in the concentration of HPMC E15. Moisture absorption of the film plays a promising role to decide the storage conditions and packaging of the films. This was alternatively affect the product stability, so there is need to control the moisture level in the developed films (Rani and Singh, 2018).

Swelling index

Swelling of the polymers is required for the bioadhesion. Degree and rate of swelling play a key role in controlling the release of the drug and these parameters are indicators for the bioadhesion and drug release profiles.Measuring swelling or degree of hydration of the polymeric film plays an important role in providing key information on the bioadhesive strength. More hydration is the reasons for relaxation and interpenetration of the polymeric chain. So, overhydration or swelling results in increase in bioadhesion strength. The swelling index for formulations F11 and F12 was found to be 12.30 and 14.61 respectively (Table 3 and Fig. 4). The formulation F12 showed higher swelling index due to increase in plasticizer. The increase in the concentration of plasticizer enhances the hydrophilic power of films leading to more swelling. On contact with water, the plasticizer gets dissolved creating porous films and hence making easy entry of water for drug dissolution (Labibet al., 2014).

In vitro antibacterial activity

B. intermedius, B. gingivalis, Actinobacillus and Wolinella recta are gram-negative bacteria responsible for the periodontitis.Since suppression/destruction of these microbes are the ultimate objective of these films, they were assessed for their capacity to effectively inhibit these microbes (Ali et al., 2012). The formulation F11 showed 25.33 ± 1.24 mmzone of inhibitionafter 168 h (Fig. 5). The activity against microbes was potentiated with the increase in the concentration of drug. The zone of inhibition of F12 formulation was 26.49 ± 1.43 mm after 168 h (Fig. 5). This study indicates developed dental film retained the antibacterial activity.The F12 formulation exhibited higher zone of inhibition and can be considered as the more effectiveformulation againstStaphylococcus aureus.

In vitro release studies of DCHdental films

Physiological properties and surface morphology of the films are primarily important for the drug release from the polymer matrix. The polymer structure holds direct relationship with the drug release. Linear amorphous polymer proved the faster drug release than the partially crystalline or cross linked polymer structure. Simultaneouly, type of plasticizer influences degradation rate of the film (Labibet al., 2014; Karkiet al., 2016). There is need to deliver the optimum concentration of the drug from the film followed by penetration of drug in the biological system. However, assessment of DCHrelease from the developed dental films is essential as it is rate- determining step in the absorption process.

The dissolution profiles of DCHdental films (F11 and F12) were evaluated in phosphate buffer pH 6.8 (stimulate GCF) as depicted in Fig. 6. Dentalfilms are matrix drug delivery devices used for intrapocket delivery of drugs (Garget al., 2012).At 24 h of dissolution, the F11 formulation showed approximately 40 % drug release. F12 formulation showed more drug release than F11 formulation at 24 h of study.It was indicated in Fig. 6 that while the concentration of the HPMC was increased and eudragit was decreased in F12 formulation, the DCHrelease was increased. Increased DCHrelease rate may due to less polymer structure or less comlplexation at that polymer mixture ratio. Moreover DCHburst release was obtained from F11 and F12 until 24 h and was decreased significatly. Remaining drug was released within 144 h in sustained release rates. In 7 d (168 h) of dissolution, complete drug release was observed for the formulation of F12. The F11 formulation showed incomplete drug release (85 %) with 7 d of dissolution study.Difference factor (f_1 = 15.74) and similarity factor (f_2 = 51.23) were calculated between the formulations F11 and F12. The f_1 and f_2 values indicated equivalent amount of drug release between the formulations. The dissolution efficiency (DE) of the F12 improved from 76.53% to 63.25% (F11). This indicated improvement in drug release from the formulation F12 than F11. There was no significant difference betweenF11 and F12 formulation (t- test, $p > 0.05$). This indicates F12 formlation more controlled drug release than F11.

Drug kinetics

Kinetics models were applied to the dissolution profiles of DCHdental films (F11, F12) and compared to find out the most probable kinetics of drug release. Parameters like correlation coefficient, release rate constant and diffusion coefficient values estimated after linearization of dissolution data (Table 4). Comparing the correlation coefficient values of zero-order for F11 and F12 (0.85, 0.87) and first-order (0.96, 0.97) respectively, it was found that drug release follows first-order kinetics indicating that drug release was independent of concentration (Table 4). In formulation F11 and F12, r^2 values were found to be high in the Higuchi model. This model described about the drug release from the matrix systems. This is used as an indicator in the diffusion controlled released (Unagola and Jayasuriya, 2018). To find out the release component (n) value, the release data was analyzed as per Korsmeyer- Peppas model. Diffusional exponent of n = 0.5 can serve as an indication for the diffusion controlled drug release. Values of nbetween 0.5 to 1.0 indicates the superposition of both the phenomena (anomalous transport) (indicates swelling-controlled drug release) and in the case of delivery system with film geometry (Kilicarslanet al., 2018). The ‘n' value of F11 and F12 batch was found to be 0.586 and 0.602 respectively. This type of release behavior was generally exhibited by controlled drug delivery systems solvent uptake determines the swelling rate of the polymer and drug release.

The present study succeeded in the formation of DCHusing the HPMC E15, eudragit RS 100, PEG 400 and dibutyl phthalate adapting solvent casting technique. The FTIR characterization showed compatibility with drug and polymers. The physiochemical properties shown by F12 film would allow to best fit into the periodontal pockets and flexibility of the films helps to avoid discomfort in the periodontal pocket. In vitroantibacterial study demonstrated inhibition of microorganism. The in vitro release of the F12 formulation revealed the developed films were capable of delivers sufficiently DCHin a sustained manner for 7 d. Conclusively, the developed doxycycline films could be considered as a promising delivery device to administer drug locally into the periodontal pockets for the treatment of periodontitis.

Table 2: Thickness, DCH content, percentage moisture loss, moisture absorption of the prepared dental films of DCH.

Formulation code	Thickness[a] (mm)	DCH content[b]	Percentage moisture loss[b]	Percentage moisture absorption[b]

F11	0.31 ± 0.01	88.33 ± 2.85	0.03 ± 0.01	0.04 ± 0.01
F12	0.35 ± 0.02	86.45 ± 1.55	0.02 ± 0.01	0.09 ± 0.02

mean ± S.D., n = 6, thickness of each film was measured at 10 different points; b. mean ± S.D., n = 3.

Table 3: Surface pH, swelling index and folding endurance of the prepared dental films of DCH.

Formulation code	Surface pH	Work of adhesion: Area[a] (N·mm) ± S.D.	Detachment force: Peak force[a] (N) ± S.D.	Swelling index	Folding endurance[a] number ± S.D.
F11	6.52	0.17 ± 0.07	1.64 ± 0.11	12.30	198 ± 3
F12	6.83	0.21 ± 0.05	1.98 ± 0.09	14.61	222 ± 3
a. mean ± S.D., n=3					

Table 4: Kinetic value of the prepared dental films (f11 and f12).

Formulation code	Zero order		First order		Higuchi		Kosmeyer-Peppas plot		Hixon Crowel plot	
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	n	R^2	K_0
F11	0.85	0.483	0.96	0.004	0.975	6.840	0.957	0.586	0.943	0.012
F12	0.87	0.489	0.97	0.005	0.97	7.220	0.938	0.602	0.952	0.013

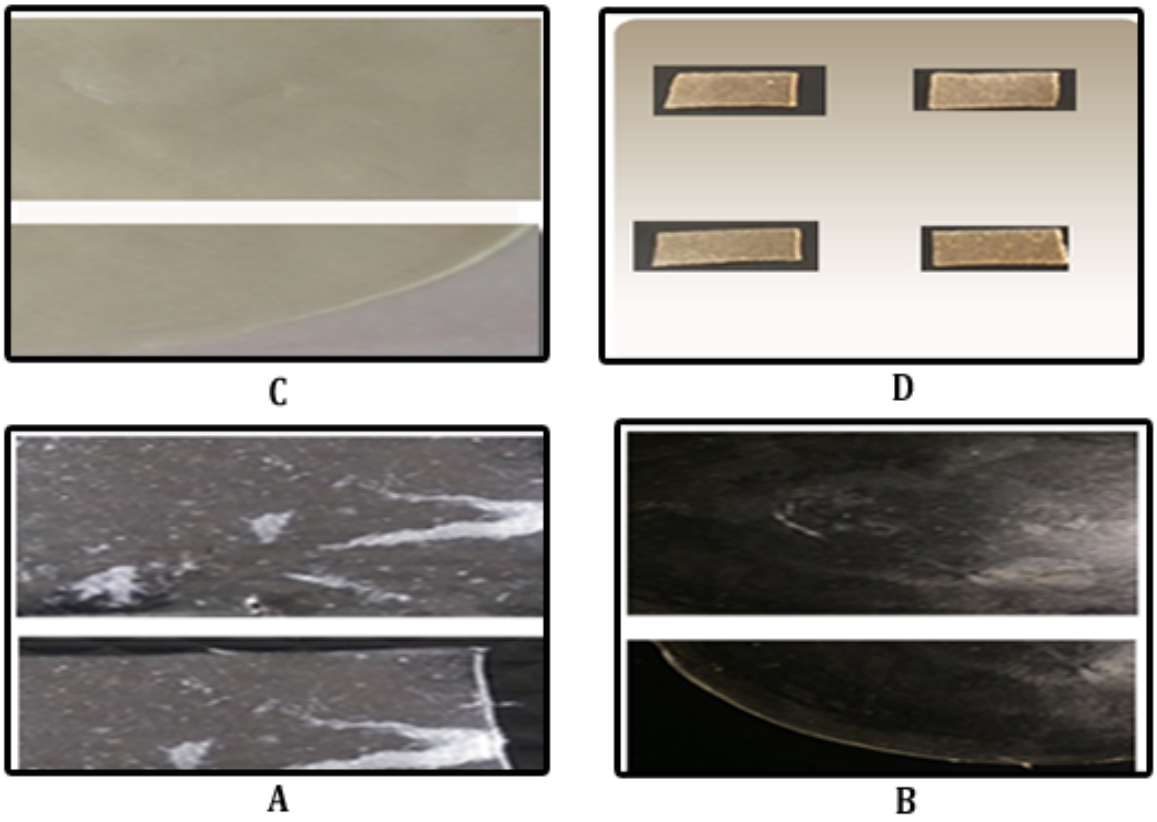

Fig. 1:Dental films of DCH (A) formulation F1, (B) formulation F11, (C) Formulation F12 and (D) dental films (F12) resized into 7 x 4 mm size.

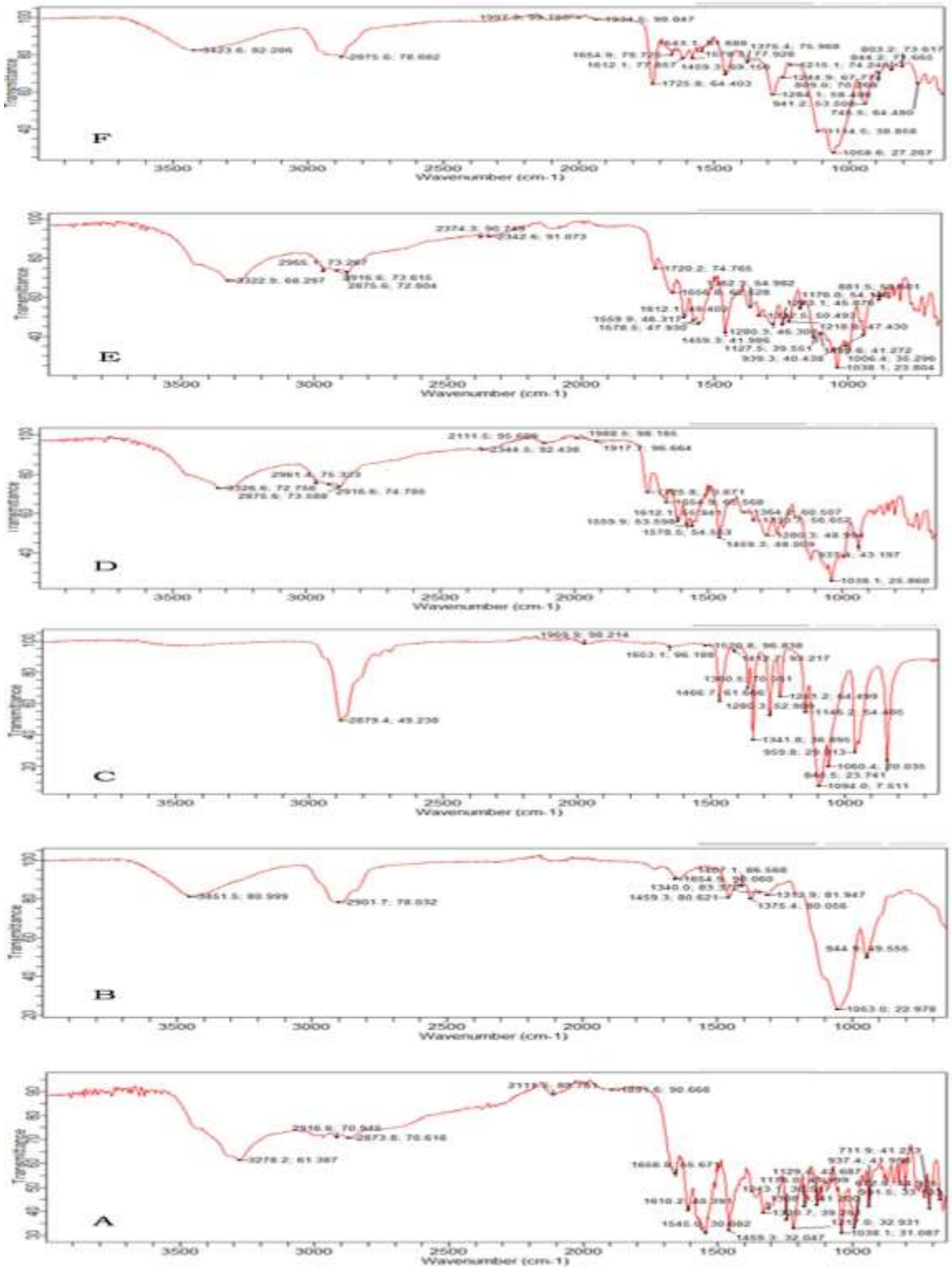

Fig. 2:FTIR spectrum of (A) DCH, (B) HPMC, (C) eudragit, (D) dibutyl phthalate, (E) PEG 400, (F) optimized formulation (F12).

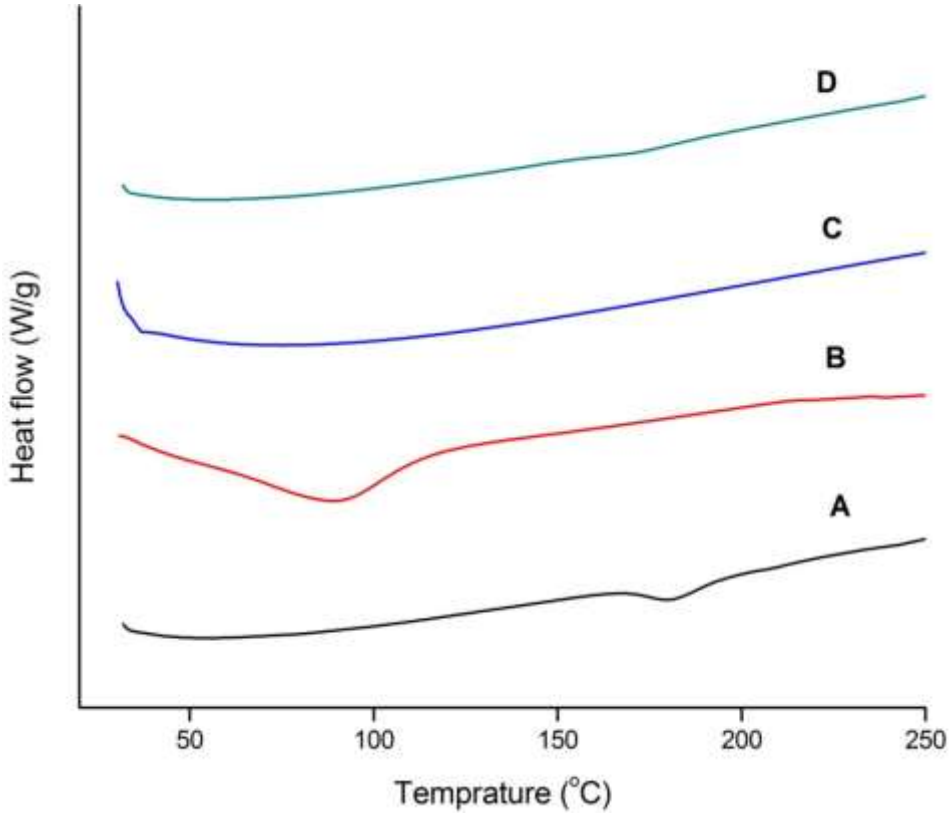

Fig. 3:DSC thermograms of (A) DCH, (B) HPMC, (C) eudragit, (D) optimized formulation (F12).

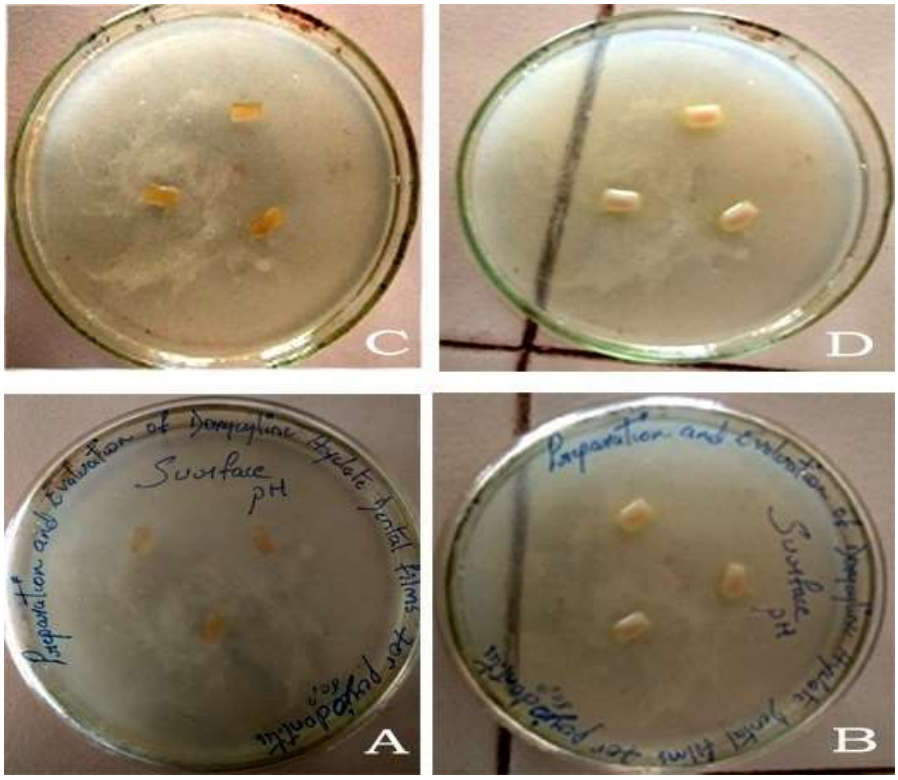

Fig. 4: Swelling study of F11 formulation (A) before swelling, (B) after swelling; F12 formulation (C) before swelling, (D) after swelling.

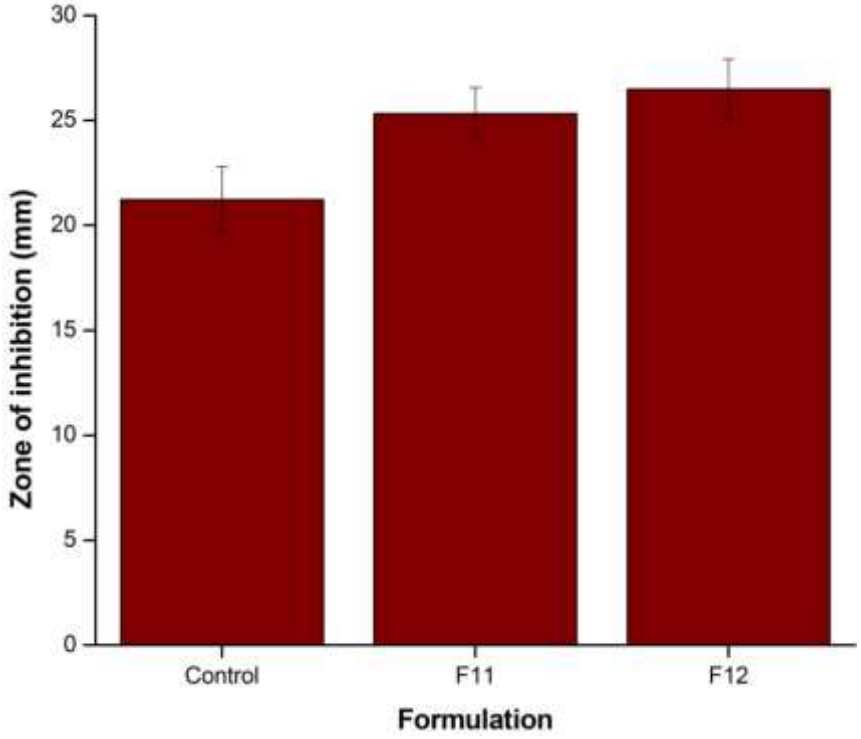

Fig. 5: In vitro antibacterial activity of DCH dental films of F11 and F12 formulations.

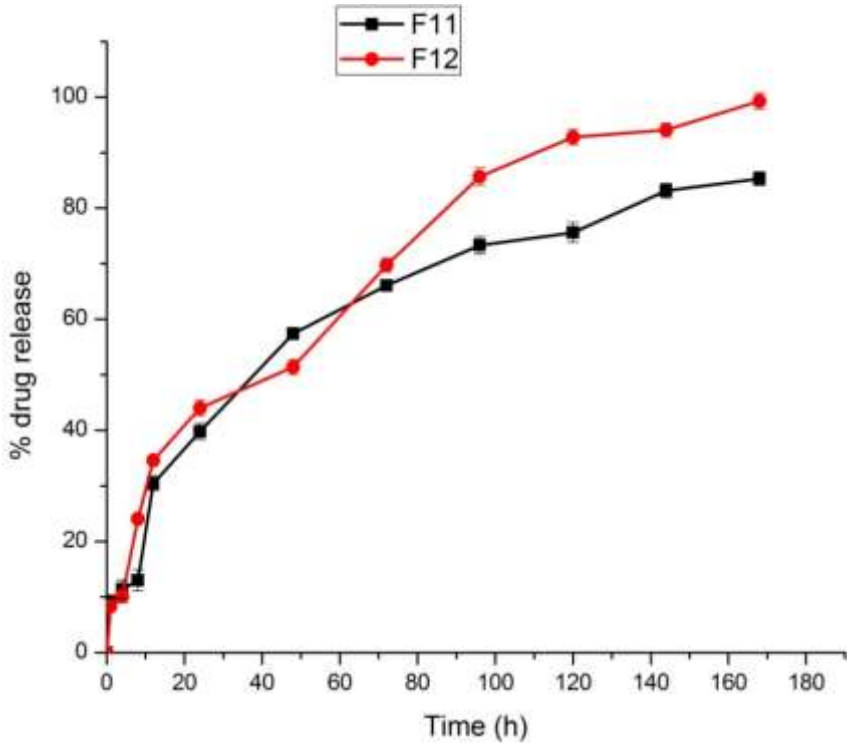

Fig. 6: In vitro dissolution profiles ofDCH dental films (F11 and F12).

CONCLUSION

In the present study we attempted to load DCH in the PMC E15 and eudragit RS 100 for local therapy of periodontitis and characterize the prepared films. After evaluating these for various parameters we concluded that it is an excellent system for drug delivery. The films were smooth, homogenous, non-sticky and flexible. The films were capable of maintaining therapeutic concentrations (above the MIC for causative organisms) for a period of 7 d. The films were capable of inhibiting the growth of aerobic and anaerobic strains commonly found in

periodontal disease. Films were developed to a satisfactory level in terms of drug content, drug release, mechanical properties, in vitro release and microbiological evaluation. Since the drug release occurred locally, it had high benefit to low risk ratio as compared to systemic administration. Hence low dose site-specific films are a better alternative. This combination of HPMC E15 and eudragit RS 100 can be used to develop dental films for other drugsWe also plan to study the effect of our device in patients after obtaining permission from ethical board.

CONTRIBUTIONS

Ranjit Prasad Swain and Abhishek Bhattacharjee: Concepts, design, literature search,data analysis, manuscript preparation, Statistical analysis, manuscript editing

Mohammad Sabira Unnisa, Santosh Kumar Dash, Ashutosh Padhan and Nabin Karna: Experimental studies, data acquisition, manuscript preparation

Malaya Ranjan Swain: Concepts, data analysis, manuscript preparation, manuscript editing

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Conflict of interest statement

The authors declare no conflict of interest.

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