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Original Article

Formulation and evaluation of gel containing extract of *Camellia sinensis* for treatment of periodontitis

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ABSTRACT

This study was designed to develop and evaluate gel containing extract of *Camellia sinensis* for treatment of periodontitis. *Camellia sinensis* leaves extraction was done by simple percolation method. Gels containing extract of *Camellia sinensis* were prepared by using various gelling agents like Carbopol 934, Carbopol 940 and xanthan gum. The gels were then evaluated for their various physicochemical properties, drug content, *in- vitro* drug release, kinetic study and anti-microbial susceptibility study. All prepared formulations showed acceptable results for all the parameters. Best formulation in terms of cumulative percent drug release (91.89 % at the end of 12 hours) along with % drug content (93.4%) was found to be F3 with acceptable bioadhesion strength (7.5gm). Formulation F3 showed comparable antimicrobial activity with marketed formulation Metrogyl. It was concluded that gel containing the *Camellia sinensis* extract can be formulated using above mentioned gelling agents. All formulations shown to have acceptable drug release and bioadhesion property.

Key words: *Camellia sinensis*, Periodontal disease, bioadhesion, Carbopol

INTRODUCTION

Periodontitis is the common oral disease affecting many people around the world. It is defined as an inflammation and progressive destruction of the tooth-supporting structures (periodontium). One of the clinical features of the periodontal disease is the formation of a periodontal pocket, which is pathologically deepened sulcus. In normal sulcus, the gap between the gingiva and the tooth is normally 1 - 3 mm deep. However, during periodontitis, the depth of pocket usually exceeds 5mm. Various Gram-negative anaerobic bacteria have been involved with the initiation of periodontal disease. *Porphyromonas gingivalis* is a Gram-negative, anaerobic, black-pigmented species which colonizes the subgingival region. It is an opportunistic pathogen which intensively participates in the initiation and progression of periodontal disease [1-3].

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Appropriate therapy for patients with periodontitis varies considerably with the extent and pattern of attachment loss, local anatomical variations, type of periodontal disease, and therapeutic objectives. The primary objectives of therapy for patients with chronic periodontitis are to halt disease progression and to resolve inflammation. The local delivery of a drug via the oral cavity mucosa is easier than transdermal drug delivery. Local delivery has the advantage of achieving higher concentrations of the drug to the intended site of action using lower dosage with an associated reduction in side effects and toxic effects. For example, dentifrices, mouth rinses, dental gels, irrigation devices and syringes. Ointments and lotions can only be applied transiently because they are diluted and removed by the saliva and sulcular fluid. Therefore, new formulations prepared by using hydrophilic polymers such as Carbopol showing excellent bioadhesion are required. A gel can be crosslinked by chemical bonds, ion interactions, hydrogen bond, or hydrophobic interactions [4,5].

Transport of materials across the oral mucosa: The majority of the drugs move across the epithelia, by passive mechanisms, which are governed by the laws of diffusion. In the case of simple diffusion, two potential routes are the paracellular (intercellular space) and transcellular pathways (across the cells) [6].

Mechanism of Bioadhesion: The process of bioadhesion occurs in two steps. First the intimate contact between the polymer and membrane followed by the formation of bonds. The bonding occurs chiefly through both physical and mechanical bonds results from entanglement of the adhesive material and the extended mucus chains. Secondary chemical bonds may be due to electrostatic interactions, hydrophobic interactions, hydrogen bonding and dispersion forces [6].

MATERIALS AND METHODS

Materials: *Camellia sinensis* leaves were obtained from local market, Mangalore and the authentication was done by Dr. Sunil Kumar, Dept. of Pharmacognosy, S.D.M, Research institute Udupi, Karnataka**.** Carbopol 934, Carbopol 940, xanthan gum and aspartame were obtained from Yarrow Chemical Mumbai, India. Methyl paraben, propyl paraben and tri ethanol amine were obtained from CDH, New Delhi, India. Peppermint oil was obtained from Loba chemicals.

Extraction of Camellia sinensis: Extraction was carried out by simple percolation method by using 70% ethanol as solvent [7].

Minimum Inhibitory Concentration (MIC) of Camellia sinensis extract: Mueller Hinton agar plates were initially spread with *Streptococcus mutans* and kept in incubator at 37˚C for around 24 hours. Subsequently the plates were removed and using a Cork Borer, four wells were made in the plates. The Herbal extracts were suitably diluted by using distilled water to get concentrations of 1mg/ml, 0.5mg/ml, 0.25mg/ml and 0.1mg/ml. Then each well was loaded with the extract of above concentrations and plate was incubated at 37˚C for around 24 hours and the zone of inhibition was measured**.**

Preparation of gel containing Camellia sinensis extract: Weighed quantity of gelling agent was dispersed in 50ml of the distilled water in a beaker and kept the beaker aside for half an hour to allow the gelling agent to swell. Then this was stirred in a homogenizer to form gel. Required quantity of methyl paraben and propyl paraben were dissolved in 5 ml of distilled water, by heating on water bath. Required quantity of *Camellia sinensis* leaves extract was mixed to the above mixture. Finally tri ethanolamine was added drop wise to the formulation for adjustment of required pH (6.8-7) and to obtain the gel at required consistency [8]. The details of different formulations are given in Table No 2.

Evaluation:

Drug polymer compatibility study

The Fourier Transform Infrared (FTIR) spectrum of pure extract was seen in between 4000 to 400 cm^{-1}. Drug-polymers compatibility studies were carried out using Fourier Transform infrared spectrophotometer. The study was carried out on individual pure extract and its physical mixture with the polymers used in the study. The IR spectrum of the physical mixture was compared with those of pure extract and peak matching was done to detect any appearance or disappearance of peak [9].

Physical examination: Prepared gels were inspected visually for their appearance, homogeneity and consistency [10].

pH measurement: pH of the prepared gel was determined by using digital pH meter. 2.5gm of the gel was dissolved in 25ml of the distilled water and pH was determined [10].

Viscosity: Viscosity of the gel was determined by using Brookfield viscometer (LVDV-II). Required quantities of the gels were taken in a beaker and spindle number 61 was used at rpm of 6 and the viscosity was noted [10].

Spreadability: To determine the spreadability, approximately 1gm of the gel was placed between two glass slides of same dimensions (20cm*20cm). A weight of 1000gm was placed on the upper glass slide and allowed the gel to spread. After 1min the weight was removed, and diameter of spread area was noted [11].

Extrudability: The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and weight of 1kg was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The % of gel extruded was calculated, recorded [12].

Bioadhesive strength measurement: The apparatus used for this study consisted of a modified double beam physical balance in which a lighter pan had replaced the right pan and left pan had been replaced by glass slide (4cm length and 2.5cm width) with plastic hang. Both sides of the balance were made equal by adding 5gm weight to the right hand pan. The height of the total setup was adjusted to accommodate a glass container.

In order to find out the bioadhesion strength, gel formulation was stacked on the glass slide with the help of adhesive. 5gm of the weight from the right hand pan was removed. This lowered the glass slide along with the gel over the membrane with a weight of 5gm. This was kept undisturbed for 5min. Then weights on the right hand side were slowly added in increments of 0.5gm till the gel just separated from the membrane surface. The excess weight on the right pan was taken as measure of bioadhesive strength [13].

Drug content: 2gm of the gel was dissolved in 10ml of phosphate buffer pH 6.8 by stirring in magnetic stirrer for 6-7 hours and filtered. Absorbance was measured at 672nm by using UV spectrophotometer.

In-vitro drug diffusion study: Drug diffusion study was performed using Franz diffusion cell employing a cellophane membrane. The membrane was initially soaked in pH 6.8 phosphate buffer solution for 24 hrs and then clamped between donor and receptor compartments of Franz diffusion cell. The phosphate buffer of pH 6.8 was filled in the receptor compartment and was magnetically stirred throughout the experiment. In the donor compartment, appropriate amount of the formulation was placed. Aliquots (5 ml) of sample were withdrawn from the receptor compartment at time intervals for 12 hours and were replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed for drug concentration using UV-Visible double beam Spectro photometer [10].

In-vitro antimicrobial susceptibility study: The agar-well diffusion method was used to determine the antimicrobial activity of the formulated gels. Mueller Hinton agar media was mixed with 24 hrs old culture of *Streptococcus mutans* and poured into sterile culture plates and allowed to solidify. With the aid of sterile cork borer, 2 wells were punched on the plate. Formulation containing the *Camellia sinensis* extract and marketed formulation (Metrogyl) were aseptically transferred into the wells and plate was incubated at 37˚C for 24 hrs. Then zone of inhibition was measured at interval of 24hrs for 3 days and both zones were compared [14].

RESULTS

The prepared gel formulations were viscous in nature and they were greenish brown in colour. All the formulations found to have good and excellent homogeneity and acceptable consistency.

The FTIR study was performed to determine the interaction between the extract and polymers. Results obtained is shown in figure 2. MIC of the extract was 0.25mg/ml with an inhibitory zone of 12mm. Obtained Zone of Inhibition for other concentrations of the extracts are shown in table 1 and figure 1. The pH values found within the range of neutral pH. Viscosity values were found within the range of 135600 – 152500cps. All prepared formulations were evaluated for spreadability, extrudability and bioadhesion. Results obtained are shown in table 3. Formulation F3 showed highest % drug content (93.4 %) and % drug release (91.89%). Details of drug release study are shown in table 3 and figure 3. *In-vitro* anti-microbial study showed that prepared formulation has comparative activity (with zone of 11mm) to that of marketed formulation (12mm). Details of anti-microbial study are shown in table 4 and figure 4.

DISCUSSIONS

From the present study it was observed that all different concentrations of the gelling agents used, resulted in satisfactory formulations. From the FTIR spectra it was concluded that there is no considerable interaction between the extract and polymers. The pH values found within the range of neutral pH which is acceptable to apply to the gums because the pH of the oral cavity is 6.8. Differences in the values of viscosity were due to the differences in the hygroscopicity, type and concentration of the gelling agents used. Spreadability and extrudability of the formulations

found to increase as the concentration of gelling agent was decreased. Bioadhesion was found maximum for the formulation F4 due to the polymer Carbopol 940 (2%). The % drug content and % drug release were maximum for F3 formulation due to lower concentration of the polymer Carbopol 934.

CONCLUSIONS

The designed formulations exhibited satisfactory physico-chemical properties, release profile and adequate drug stability. Over all the formulations, F3 showed most satisfactory results for the parameters evaluated.

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Table 1: Zone of inhibition of various concentrations of *Camellia sinensis* extract

Concentration of the Extract (mg/ml)	Zone of Inhibition (mm)
1	21
0.5	14
0.25	12
0.1	Nil

Table 2: COMPOSITION OF THE HERBAL GEL:

Ingredients in % w/w	Formulation Code							
	F1	F2	F3	F4	F5	F6	F7	F8
Camellia sinensis extract	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Carbopol 934	1.0	2.0	-	-	0.5	1.0	-	-
Carbopol 940	-	-	1.0	2.0	0.5	1.0	-	-
Xanthan gum	-	-	-	-	-	-	1.0	2.0
Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Aspartame	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Peppermint oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Triethanol amine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water q.s	100	100	100	100	100	100	100	100

Table 3: EVALUATION OF THE PREPARED GEL FORMULATIONS.

Formulation code	pH	Viscosity (cps)	Spreadability (cm)	Extrudability (%)	% drug content	Bioadhesion strength (gm)	% CDR
F1	6.81	148800	5.2	84.4	91.2	6.5	89.50
F2	6.76	149200	4.9	80.2	89.35	7.0	86.69
F3	6.82	152300	5.0	83.5	93.4	7.5	91.89
F4	6.83	152500	4.8	81.6	90.3	8.0	87.71
F5	6.77	143800	5.2	84.3	88.5	6.5	86.97
F6	6.79	148500	5.1	78.7	88.1	7.0	85.03
F7	6.75	135600	5.4	85.3	86.7	6.0	83.90
F8	6.78	138900	5.3	84.8	86.2	6.5	83.10

Table 4: IN-VITRO ANTIMICROBIAL ACTIVITY

Formulations	Zone of inhibition (mm) after 72 hours
F3	11
Marketed formulation (Metrogyl)	12

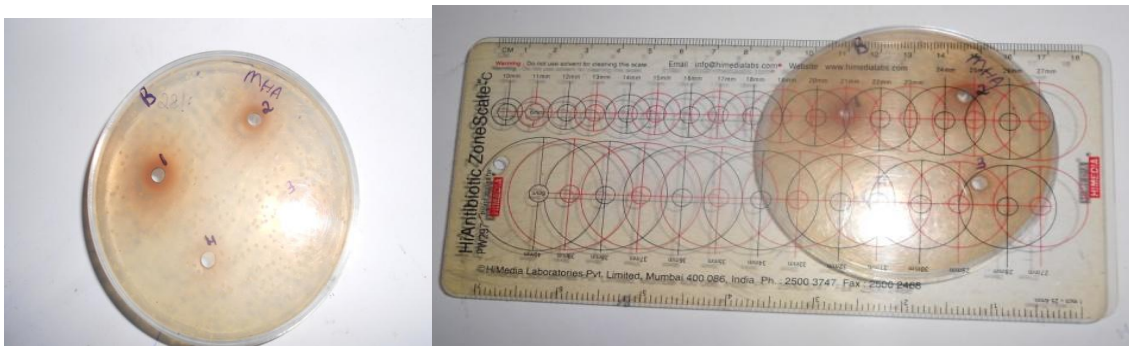

Fig.1: Zone of inhibition of various concentrations of *camellia sinensis* extract

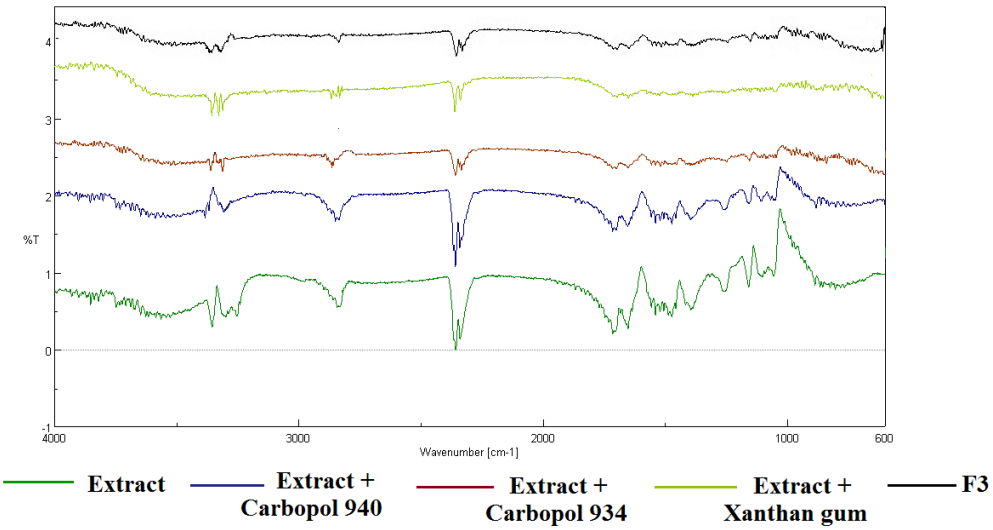

Figure 2: FT-IR spectrum of extract and polymer combinations

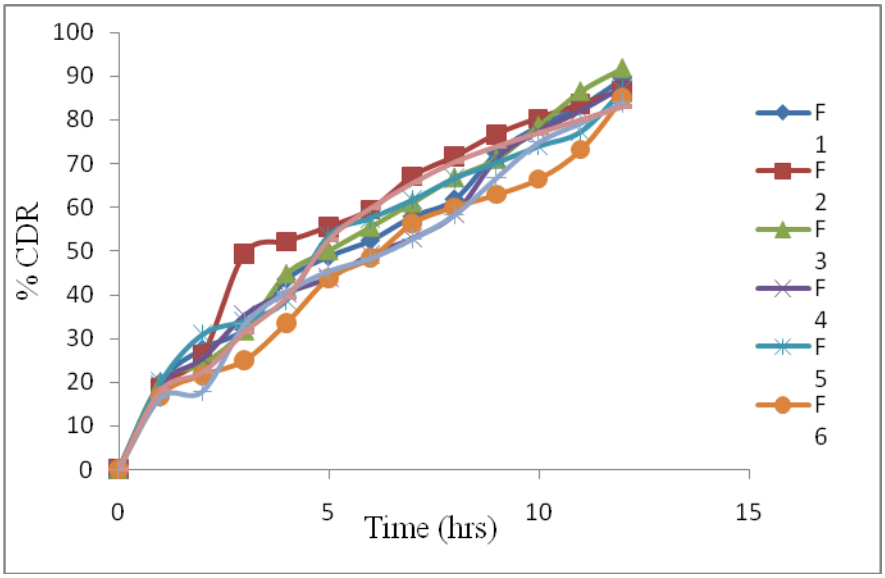

Figure 3: Release profile of all formulations.

Figure 4: Anti-microbial activity of Metrogyl and F3

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