Original Article

Design, formulation and evaluation of a mucoadhesive gel from *Quercus brantii L.* and *coriandrum sativum L.* as periodontal drug delivery

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Abstract

Background: Periodontitis is inflammation of the supporting tissues of the teeth caused by specific microorganisms. Intra-periodontal pocket, mucoadhesive drug delivery systems have been shown to be clinically effective in the treatment of periodontitis. The aim of this study was to formulate a mucoadhesive gel from the seed hull of *Quercus brantii* and fruits of *Coriandrum sativum* for the treatment of periodontitis.

Materials and Methods: The semisolid concentrated extracts were incorporated in gel base. Mucoadhesive gels were prepared using carbopol 940, sodium carboxymethylcellulose (sodium CMC) and hydroxypropyl methylcellulose K4M (HPMC) as bioadhesive polymers. Physicochemical tests, mucoadhesive strength measurement and *in vitro* drug release study were carried out on two formulations containing carbopol 940 and sodium CMC polymers (Formulations F_4 and F_5). We investigated the antibacterial activity of formulation F_5 against *Porphyromonas gingivalis* using the disk diffusion method on supplemented Brucella agar.

Results: Eight gel formulations were prepared. Physical appearance, homogeneity and consistency of F_4 and F_5 were good. Mucoadhesion and viscosity of F_5 (1% carbopol 940 and 3% sodium CMC) was more than F_4 (0.5% carbopol 940 and 3% sodium CMC). Drug release from F_5 was slower. Both of formulations were syringeable through 21 G needle. In the disk diffusion method, F_5 produced significant growth inhibition zones against *P. gingivalis*.

Conclusion: The ideal formulation for the treatment of periodontitis should exhibit high value of mucoadhesion, show controlled release of drug and be easily delivered into the periodontal pocket preferably using a syringe. Based on *in vitro* release and mucoadhesion studies, F_5 was selected as the best formulation.

Key Words: *Coriandrum sativum*, herbal gel, periodontal drug delivery, polyphenol, *Quercus brantii*

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INTRODUCTION

Periodontitis is inflammation of the supporting tissues of the teeth caused by specific microorganisms. One of the most important clinical signs of periodontal disease is periodontal pocket. The periodontal pocket is gingival sulcus pathologically deepened. Clinical signs including gingival bleeding, suppuration,

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tooth mobility and localized pain show the presence of periodontal pocket. A main etiologic reason in periodontal disease is bacterial plaque. The gingival crevice or pocket is full of crevicular fluid and contains many materials that bacteria may use as nutrients. There are high percentages of anaerobic (90%) and gram-negative (75%) bacterial species in chronic periodontitis. The counts of bacteria in a periodontal pocket are from 10^3 bacteria in a healthy crevice to greater than 10^8 bacteria in a deep pocket. The major pathogen of chronic periodontitis is *Porphyromonas gingivalis*, a gram-negative anaerobic black-pigmented bacterium. The most important approach for periodontal treatment is the control of inflammation, carried out partly by plaque and calculus removal in scaling and root planing.[1]

Scaling and root planing is not always adequately effective. This has led to the adjunctive use of antibiotics, usually in the form of a local delivery system.[2] Because periodontal infections may contain a wide variety of bacteria, no single antibiotic is effective against all putative pathogens. Now, an ideal antibiotic for the treatment of periodontal disease does not exist.[1] In periodontitis, antioxidant activities of saliva and crevicular fluid decrease. Therefore, the use of antioxidants may inhibit periodontal disease development.[3] Intra-periodontal pocket, mucoadhesive drug delivery systems have been shown to be clinically effective in the treatment of periodontitis.[4] Periodontal pocket is able to retain a delivery system for a desired period of time.[5] Natural herbs can help stop or reverse the development of periodontitis.[6] Plants are cost-effective and have fewer side-effects.[7] It would be expected that local delivery of an herbal product into periodontal pocket as an adjunct to scaling and root planing, which has antibacterial, antioxidant activities, anti inflammatory and analgesic effects and also can control bleeding on probing would be advantageous.

Polyphenols are plant metabolites.[3] Tannins are a family of polyphenols that precipitate proteins.[8] Polyphenols exhibit *in vitro* antibacterial activity against periodontal pathogens.[3] Tannins precipitate microbial proteins and prevent the development of microorganisms.[9] Polyphenols increase antioxidant ability of oral fluids and prevent from periodontal disease.[3] Tannins are free radical scavengers and have antioxidant activity.[10] Polyphenolic compounds, locally, by creating an impenetrable layer with protein or polysaccharide may decrease inflammation. In a study, anti-inflammatory activity of tannins was evaluated and the results indicated that tannins decrease the inflammatory response by scavenging activities. Tannins inhibit inflammatory markers via oxidation of the tannin and reduction of free radicals.[8] Tannins are proved hemostatics.[11]

Dietary intake of polyphenols could be as high as 1 g/day.[12] In the human saliva, proline-rich proteins with polyphenols form stable complexes, which remain stable during gastrointestinal tract and are not systemically absorbed and do not cause side-effects.[3,13]

Polyphenols and tannin are the main substances in oak tree.[14] In all parts of the Zagros region in the west of Iran, *Quercus brantii* is abundant species.[15] *Quercus brantii* is from the fagacea family.[14] Oak was used traditionally to treat hemorrhoid, varicose veins, diarrhea, gastric ulcers, superficial injuries, local inflammation.[14,16] *Quercus brantii* has hemostatic effect,[11] antibacterial activity,[7,17] anti-inflammatory and anti-nociceptive effects,[18] and antioxidant activity.[19] Oak effects could be due to polyphenols and tannins.[14]

Coriandrum sativum is from Umbelliferae family and is widely cultivated in Iran. The fruits of *C. sativum* were used in Iranian folk medicine as carminative and spasmolytic.[20] *C. sativum* is said to have anti-inflammatory activity,[21] analgesic effect, antibacterial activity,[22] and antioxidant activity.[23] Linalool is the major substance of essential oil in *C. sativum*.[21] *C. sativum* extract has also tannin.[22]

A significant characteristic of the oral gel is mucoadhesive strength for adhesion to the mucosa in the dental pocket. Good gel adhesion to the mucosal surface results in prolonged residence time and contact time and better clinical efficacy.[24] Beside increase the residence time and contact time of gel with the mucosa, drug release from the gel must be controlled.[25] Gels are able to control drug release.[26]

Locally delivered antimicrobial agents are available as adjuncts to scaling and root planing including subgingival chlorhexidine, tetracycline-containing fibers, subgingival doxycycline, subgingival minocycline, subgingival metronidazole.[1]

The aim of this study was to formulate a subgingival mucoadhesive gel from the seed hull of *Q. brantii* been locally known as Jaft and fruits of *C. sativum* as an adjunct to scaling and root planing for the treatment of periodontitis, which has antiseptic, anti-inflammatory and analgesic effects and for the control of bleeding on probing.

MATERIALS AND METHODS

Collection and identification of plant materials

Dried fruits of *C. sativum* were obtained from a local market in Isfahan province of Iran. The fruits of *Q. brantii* were collected from suq in Kohgiluyeh and Buyer Ahmad province of Iran during summer. The fruits were washed and dried in air and seed hulls (Jaft) separated. The plant specimens identified by Dr. Iraj Mehregan in Tehran Islamic Azad University.

Chemicals

Folin-Ciocalteu reagent, gallic acid, sodium carbonate anhydrous, carbopol 940, sodium CMC, HPMC K4M, polyethylene glycol 400 (PEG 400), methyl paraben, propyl paraben, and triethanolamine were obtained from Sigma-Aldrich Chemie Gmbh, (Steinheim, Germany). Sodium alginate was obtained from BDH chemicals Ltd., (Poole, UK).

Preparation of extracts

Percolation method was used for extraction. Hundred gram plant materials (Jaft of *Q. brantii* and the fruits of *C. sativum*) were powdered and soaked in 300 ml ethanol 70%. After 2 h, the plant materials were extracted with ethanol 70% (700 ml) by percolation method. After 48 h, extracts were concentrated by rotary evaporator (Heidolph VV 2000). Further, concentration was done over the boiling water bath.[11]

Determination of pH of the extracts

pH of extracts was measured using digital pH meter (Metrohm 632, Swiss). The measurement of pH was performed immediately after concentration of the extracts and 48 h, 1 week, 2 weeks, and 4 weeks after concentration of the extracts. The measurement of pH of each sample was done in triplicate and average values are calculated.[11]

Total phenolic content

Total phenolic content was determined according to the Folin-Ciocalteu method.[27] Gallic acid was used as standard and total phenolic content expressed as mg of gallic acid equivalents (GAE) per gram.[16] For preparation of gallic acid stock solution, in a 100 ml volumetric flask, 500 mg of dry gallic acid in 10 ml of ethanol 96% was dissolved and diluted to volume with water. For preparation of calibration curve, 0, 1, 2, 3, 5 and 10 ml of the phenol stock solution were added into 100 ml volumetric flasks, and then diluted to volume with water. These solutions will have phenol concentrations of 0, 50, 100, 150, 250 and 500 mg/L gallic acid.[27] For preparation of sample solution, Jaft powder of *Q. brantii* and fruit powder of *C. sativum* (10 g) were added to 250 ml boiling water and extracted under continuous stirring for 10 min. Then the extracts were filtrated and concentrated using rotary evaporator. The concentrated extracts were lyophilized by freeze dryer (Snijders Tilburg 2040, Holland) and dry extracts were obtained.[16,28] 100 mg dry extract of plants is dissolved in 15 ml of hot water and filtrated.[11]

Folin-Ciocalteu test: Briefly, 1.58 ml water was added to each calibration solution, sample and blank (20 µl) and then 100 µl of Folin-Ciocalteu reagent was added and well mixed. After 30 seconds, 300 µl of 20% sodium carbonate solution was added and the sample tubes were left at room temperature for 2 h. The absorbance of each solution was determined at 765 nm against the blank with a UV-VIS spectrophotometer (UVmini 1240, Shimadzu) and plot absorbance vs. concentration. For *C. sativum*, 20 µl was added as for the calibration solutions, but in the case of *Q. brantii*, the sample was diluted by 10 first, then 20 µl was added.[27]

Preparation of gel formulations

For preparation of gel formulations, semisolid concentrated extracts of the seed hull (Jaft) of *Q. brantii* and fruits of *C. sativum* were used. Carbopol 940, sodium CMC and HPMC polymers were used as gelling agent.

Carbopol 940 gel

Methyl paraben and propyl paraben were dissolved in water 80°C.[29] Accurately weighed quantity [Table 1] of carbopol 940 was dispersed in water 40°C with constant stirring using mechanical stirrer at 1200 rpm for 30 min. The extracts were dissolved in PEG 400 and added to the base and well mixed. The pH was then adjusted to pH, 6 using triethanolamine and stirred slowly until a clear gel was obtained.[26,30]

Sodium CMC gel

Methyl paraben and propyl paraben were dissolved in

Table 1: Composition of gel formulations with different polymers (Carbopol 940, sodium CMC, HPMC)

Ingredients (g)	Formulations							
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Carbopol 940	0.5	1	-	0.5	1	-	-	-
Sodium CMC	-	-	3	3	3	-	-	-
HPMC	-	-	-	-	-	2	3	5
Q. brantii extract	20	20	20	20	20	20	20	20
C. sativum extract	1	1	1	1	1	1	1	1
PEG 400	13	13	13	13	13	13	13	13
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Triethanolamine	qs	qs	-	-	-	-	-	-
Water qs to	100	100	100	100	100	100	100	100

water 80°C.[29] Accurately weighed quantity [Table 1] of sodium CMC was dispersed in water 50°C with constant stirring using a mechanical stirrer at 2000 rpm for 30 min. The extracts were dissolved in PEG 400 and added to the gel base and mixed well to get a homogenous gel.[30]

HPMC gel

Methyl paraben and propyl paraben were dissolved in water 80°C.[29] Accurately weighed quantity of HPMC [Table 1] was dispersed in a portion of hot water (about one third of the total volume) heated at 80°C with constant stirring using a mechanical stirrer. Stirring was continued until a thin hazy dispersion was formed, then the remaining amount of water was added on cold and mixing was continued till smooth homogenous gel is formed. The gel was left overnight in the refrigerator (hot/cold technique). The drug solution in PEG 400 was added to the gel base and mixed well to get a homogenous gel.[31]

Carbopol 940 and sodium CMC gel

Methyl paraben and propyl paraben were dissolved in water 80°C.[29] Accurately weighed quantity [Table 1] of carbopol 940 and sodium CMC were dispersed in water separately as was explained above and were mixed well. The extracts were dissolved in PEG 400 and added to the gel base and mixed well.

Evaluation of gel formulations

Physical appearance of gel formulations

Gel formulations were visually inspected for clarity, color, homogeneity, consistency and presence of particles.[26] Homogeneity was examined by microscope.[11] In order to investigate the consistency of the formulations, a small quantity of gel was pressed between the thumb and the index fingers and the consistency of the gel was noticed.

Determination of pH in gel formulations

pH was measured in each gel, using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4 and 7. One gram each of the gel formulations was accurately weighed and dispersed in 10 ml of purified water. The electrode was inserted into the sample 10 min priors to taking the reading at room temperature. The measurement of pH of each formulation was done in triplicate and average values are calculated. The measurement of pH was performed at 48 h, 1 week, 2 weeks, 1 month, 3 months and 6 months after preparation to detect any pH fluctuation with time.[32]

Centrifugal test

In order to investigate the stability of the formulations against the centrifugal force, 48 h after preparation, formulations were transferred into tubes and centrifuged at 2000 rpm for 60 min, using a centrifugal device (Centrifuge 5430) and stability of formulations were evaluated at the times of 5, 15, 30 and 60 min.[11]

Thermal test

In order to investigate the stability of various formulations in different seasons and weather conditions, 48 h after preparation, from each formulation, 3 samples were placed at 4°C, 25°C and 45°C. Gel formulations were evaluated at the times of 24 h, 1 week, 1 month, 3 months and 6 months.[11]

Freeze and thaw test

In order to investigate the stability of the formulations in extreme cold, 48 h after preparation, from each formulation, 15 g gel was placed at -8°C for 48 h and then 48 h at 25°C for six periods. Then stability of gel formulations was evaluated.[11]

Cooling and heating test

In order to investigate the stability of the formulations against extreme temperature changes, 48 h after preparation, from each formulation, 15 g gel was placed at 45°C for 48 h and then 48 h at 4°C for six periods. Then stability of gel formulations were evaluated.[11]

Drug content determination in gel formulations

48 h after preparation, one gram gel was taken in 10 ml volumetric flask, dissolved in water, made up the volume to 10 ml with water.[33] Total phenolic content was determined according to the Folin-Ciocalteu method. Absorbance values were measured at 765 nm. Concentrations of polyphenols were calculated from standard calibration curve of gallic acid.[16,27]

In vitro mucoadhesion measurement

A significant characteristic of gel in the treatment of periodontitis is mucoadhesive strength for adhesion to the mucosa in the dental pocket. A modified tensiometry method based on Fisher's tensiometer was used to evaluate the mucoadhesive properties of gel formulations. The limitation of the measurable surface tension or adhesive forces by this instrument was set at 0-100 dyne/cm^2. A thin mica disk was placed on the tensiometer ring. 1% (w/v) solution of sodium alginate was placed into the 37°C water-jacketed glass vial of the instrument. After calibration the tensiometer with a standard weight, 200 mg from each gel formulation was placed on the mica disk and was transferred to the tensiometer. The gel formulation came in contact with the 1% (w/v) solution of sodium alginate for 5 min. Then the gel formulation was detached from the solution of sodium alginate by a 0.2 inch/min speed. The adhesion force between mica disk and the solution

of sodium alginate was used as the blank and considered in all tests. The detachment force was measured in terms of dyne/cm^2. The test was performed for six times on each formulation. After each experiment the glass plate was removed and the Palladium-Platinum ring of the tensiometer was washed with methanol, then acetone.[34]

In vitro drug release study

Diffusion studies of the prepared gels were carried out in Franz diffusion cell through a cellulose acetate membrane. Gel sample (0.5 gram) was taken in cellulose acetate membrane and the diffusion studies were carried out at 37 ± 1°C using 28 ml of purified water as the dissolution medium. 1 ml of each sample was withdrawn periodically at 0.5, 1, 2, 3, 4, 5 and 6 h and each sample was replaced with equal volume of fresh dissolution medium. Then the samples were analyzed for the drug content by using purified water as blank and concentrations of polyphenols were calculated from standard calibration curve of gallic acid. Concentrations obtained are apparent concentrations. To calculate the actual amount of drug released, a correction factor was used. This correction factor is calculated based on the following equation. With adding the correction factor to the apparent amount of drug released, the actual amount of drug released was obtained.

$$C_n = C + \frac{C_{n-1}.V}{V_t}$$

In this equation, C_n is the actual concentration of drug released in sample n, C is the apparent concentration of drug released in sample n, C_{n-1} is the actual concentration of drug released from sample before sample n, V is the sample volume, V_t is the volume of receiver phase. The actual percentages can be calculated from the actual concentrations. Then graph of the percentage of drug released was plotted versus time for gel formulations.[11]

Drug release kinetic studies of gel formulations

To study the mechanism of drug release from the formulations (F_4, F_5), drug release data were analyzed according to zero-order, first-order and Higuchi equations. The criterion for selecting the most appropriate was chosen on the basis of goodness of fit test. To investigate the mechanism of polyphenols release from gel formulations, the release data were analyzed with the following mathematical models: Zero-order kinetic (equation a), first-order kinetic (equation b), and Higuchi kinetic (equation c).

$$Q_t = K_0 t \quad \text{(a)}$$

$$\ln Q_t = \ln Q_0 - K_1 t \quad \text{(b)}$$

$$Q_t = K_h t^{1/2} \quad \text{(c)}$$

In these equations, Q_t is the percent of drug released at time t, Q_0 is the percent of drug present in the gel, K_0, K_1 and K_h are the constants of the equations. In zero-order kinetic model, diagram of cumulative % drug release is plotted versus time and a linear plot is obtained. In first-order kinetic model, log cumulative of % drug remaining is plotted versus time and a linear plot is obtained. In Higuchi kinetic model, cumulative % drug release is plotted versus square root of time and a linear plot is obtained.[35]

Determination of viscosity

Viscosity (in cps) of the prepared gels was measured by a Brookfield DV-III viscometer at 100 rpm, using spindle number 7 at 25°C. Samples of the gels were to settle over 30 min at the room temperature, before the measurements were taken.[35] The test was performed for three times on each formulation.

Syringeability study

For drug delivery into the periodontal pocket, injectable systems are useful. The use of injectable systems is easy and rapid. Syringeability of gel formulations was evaluated through 21 G needle.[33,36]

In vitro evaluation of antibacterial activity of gel formulation (F_5) against *Porphyromonas gingivalis* using the disk diffusion method

The most common etiological agent of chronic periodontitis is *Porphyromonas gingivalis*.[1] In this study, antibacterial effect of gel formulation (F_5) against *P. gingivalis* using the disc diffusion method was studied. *P. gingivalis* was prepared in lyophilized form from the Persian type culture collection of the Iranian research organization for science and technology. The lyophilized bacteria were resuscitated in the sterile conditions of the laboratory. *P. gingivalis* was cultured on supplemented brucella agar plates (prepared from the Iranian research organization for science and technology). Culture media were incubated for 72 h in anaerobic condition at 35°C in order to allow bacterial growth.[37] After bacterial growth, a microbial suspension was prepared and transferred to the plates containing culture medium by a sterile swap. Then, sterile paper discs with a diameter of 5 mm (Blank disc, Patan Teb, Iran) were smeared with gel formulation and were placed on the surface of the plates containing culture medium in equal distances. Then, the plates were incubated at 35°C for 72 h. After this period, the diameter of the zone of inhibition around the disc was measured in millimeters and the average of diameters noted. Test was conducted in triplicate. Sterile discs free of any material were used as negative control in culture medium.[38] For positive control, tetracycline disc (30 μg/ml) was used.[39]

RESULTS

Evaporation and solvent removal of hydroalcoholic extracts of *Q. brantii* and *C. sativum* gave semisolid concentrated extracts yielded 70 and 10%, respectively. The pH of extracts of *Q. brantii* and *C. sativum* used in the formulations were in the range of 6-7. The weight of dry extracts of *Q. brantii* and *C. sativum* obtained after freeze drying were 63 and 18.5%, respectively. The dry extracts of *Q. brantii* and *C. sativum* were found to contain 504.10 and 30.77 mg/g of total phenolic content, respectively. Total phenolic content was expressed as GAE mg/g using the equation obtained from the calibration curve of gallic acid ($y = 0.001x + 0.0048$, $R^2 = 0.9993$).

Semisolid concentrated extracts of *Q. brantii* (20%) and *C. sativum* (1%) were incorporated in gel base and eight formulations were prepared from carbopol 940, sodium CMC and HPMC polymers [Table 1]. Physical appearance, homogeneity and consistency of F_4 and F_5 were good. Consistency of F_1, F_2, F_3 was low. Physical appearance and homogeneity F_6, F_8 and F_9 were not good. Precipitation occurs in F_6, F_7 and F_8, which could be due to the incompatibility in the system. Hence, these formulations were discarded.

Since consistency and physical appearance of F_4 and F_5 was good, other tests were performed on them. In centrifugal test, thermal test, freeze and thaw test, cooling and heating test, F_4 and F_5 had good physicochemical characteristics and were stable in the experimental conditions. Table 2 shows the results of determination of pH, drug content, mucoadhesive strength measurement and determination of viscosity carried out on F_4 and F_5.

The results of mucoadhesive strength measurement by modified tensiometer method were shown in Table 2. Mucoadhesion of F_5 (containing 1% carbopol 940 and 3% sodium CMC) was more than F_4 (containing 0.5% carbopol 940 and 3% sodium CMC). Franz diffusion cell was used to study *in vitro* drug release. As it is seen in Figure 1 and Table 3, drug release from F_5 was slower than F_4. For F_5 the time required for release of 50% of total drug content were more (5 h) and for F_4 was 3 h. For *in vitro* release kinetic study, the dissolution profile of F_4 and F_5 was fitted to zero-order, first-order and Higuchi equations to determine the kinetic modeling of drug release. Release data of F_4 and F_5 showed R^2 value of 0.9907 and 0.9919 for Higuchi equation, respectively. For explanation of their kinetic, Higuchi kinetic model was suitable [Table 3]. The viscosity of gels was determined by a Brookfield DV-III Rheometer at 100 rpm, at 25°C, using spindle number 7. Viscosity of F_5 was more.

Drug content in F_4 and F_5 were 32.6 and 32.8 mg GAE/g, respectively. The results of syringeability study indicated that F_4 and F_5 were syringeable through 21G needle.

The antibacterial activity of F_5 against *P. gingivalis* was evaluated by the disk diffusion method on supplemented brucella agar. The growth inhibition zones were measured. After 72 h of incubation, gel formulation F_5 displayed observable zones of inhibition. The sterile filter disc as negative control produced no observable inhibitory effect for the bacteria. Table 4 shows inhibition zones diameter of F_5 and tetracycline disc against *P. gingivalis*. The results showed that the mean of inhibition zone for gel formulation was 17.23 ± 0.2 mm. For tetracycline disc as positive control, the mean of inhibition zone was 20 mm.

Table 2: Results of determination of pH, drug content, mucoadhesive strength (tensiometry method) and viscosity (at 100 rpm, 25°C) in formulations F_4 and F_5 (Mean±SD)

Physicochemical characteristics	F_4	F_5
pH 48 h after preparation	6.5±0.3	6.6±0.2
Drug content (mg GAE/g)	32.6±0.3	32.8±0.3
Mucoadhesive strength (dyne/cm^2)	9±0.5	12±0.5
Viscosity (cps)	4950±50	6950±150

DISCUSSION

Periodontal diseases are mostly associated with anaerobic gram negative rods such as *P. gingivalis*. In a periodontal pocket the bacteria form biofilm. For treatment of periodontal pockets, mechanical removal (scaling and root planing), systemic anti-infective therapy and local anti-infective therapy (placing anti-infective agents directly into the periodontal pocket) are used.[1] Many medicinal plants and their products are widely used for prevention and treatment of oral infections. Polyphenols exhibit *in vitro* antibacterial activity against periodontal

Table 3: Drug release and drug release kinetics of gel formulations (F_4 and F_5) (n=3)

Formulations	Cumulative drug release (%)	Zero-order release		First-order release		Higuchi equation	
		K_0	R^2	K_1	R^2	K_h	R^2
F4	79.78 ± 1.2 (6 h)	12.792	0.9489	0.1193	0.9739	32.576	0.9907
F5	54.29±3 (6 h)	9.1935	0.9546	0.058	0.9899	22.168	0.9919

K_0, K_1 and Kh are kinetic constants. R^2 is correlation coefficient

Table 4: Inhibition zones diameter of gel formulation F_5 against *P. gingivalis* (Mean±SD)

Bacterial species	Formulation F_5	Positive control
P. gingivalis	17.23±0.2	20.00±0.0

Numbers are mean diameter of inhibition zones in mm, Tetracycline disc (30 μg/ml) were used as reference antimicrobial compound

pathogens and increase antioxidant ability of oral fluids and also decrease inflammation.[3,8] Polyphenols are main substances in oak tree.[14] A gel with good mucoadhesive strength in periodontal pocket exhibits prolonged residence time and contact time and better clinical efficacy.[24]

The dry extracts of the seed hull (Jaft) of *Q. brantii* and fruits of *C. sativum*, according to the Folin-Ciocalteu method were found to contain 504.10 and 30.77 mg GAE/g of total phenolic content, respectively. It has been reported that total phenolic content in fruits of *Q. brantii* in Chaharmahal and Bakhtiari province of Iran is 281.55 mg GAE/g.[19] In present study, the results indicate that the seed hull (Jaft) of *Q. brantii* contains considerably polyphenols.

Semisolid concentrated extracts of *Q. brantii* (20%) and *C. sativum* (1%) were incorporated in gel base and eight formulations were prepared from carbopol 940, sodium CMC and HPMC polymers as gelling agent. Because consistency and physical appearance of F_4 and F_5 were good, both of formulations were selected for doing other tests. In centrifugal test, thermal test, freeze and thaw test, cooling and heating test, F_4 and F_5 had good physicochemical characteristics and were stable in the experimental conditions.

The results of mucoadhesive strength measurement by modified tensiometer showed mucoadhesion of F_5 (containing 1% carbopol 940 and 3% sodium CMC) was more than F_4 (containing 0.5% carbopol 940 and 3% sodium CMC) [Table 1]. The results showed that increasing the concentration of carbopol 940 could increase the mucoadhesion of the formulation. Carbopols have high-molecular weight and swell in water up to 1000 times of the original volume, so make large adhesive surface with the mucin and give good mucoadhesiveness.[26] Carbopols form secondary bioadhesion bonds with mucin, while other polymers form superficial bioadhesion.[40] Carbopols are used in formulations as mucoadhesive polymers. The addition of mucoadhesive polymers in the formulations results in increase of the contact time with mucosa and prolongation of residence time.[26] A significant characteristic of the oral gel is mucoadhesive strength for adhesion to the mucosa in the dental pocket. Good gel adhesion to the mucosal surface results in increase the contact time with the mucosa and prolongation of residence time and better clinical efficacy.[25]

Franz diffusion cell was used to study *in vitro* drug release. Drug release from F_5 was slower and provides an extended release profile. For F_5 (containing 1% carbopol 940, and 3% sodium CMC), the time required for the release of 50% of total drug content was more (5 h) and drug release was slower. For F_4 (containing 0.5% carbopol 940 and 3% sodium CMC), the time required for the release of 50% of total drug content was 3 h. It would be expected that F_5 would be potentially useful in the treatment of periodontitis. Moreover, the release of drug is expected to be slower in the periodontal pocket. The results indicated that increasing the concentration of carbopol 940 could decrease the release rate, due to increased viscosity of the formulation. Changes in the polymer mixing ratios affect considerably on the release rate. The results indicated that F_5 can retain drugs better than F_4. Hydrogels hydration and swelling are main factors in determination of drug release rate and bioadhesiveness. Hydration and swelling depend on degree of polymer crosslinking. It has been reported that degree of crosslinking in carbopol 940 is high. With polymer swelling, drug release is controlled.[41] With increase in polymer amount, the gel becomes thicker and water penetration is limited and results in reduction in drug release.[40] Beside mucoadhesive property and increase the contact time of gel with mucosa, drug release from the gel must be controlled.[25] Gels are able to control drug release.[26] Carbopols show excellent bioadhesion and can be used as a drug carrier to control the release of a drug.[42]

Viscosity of F_5 was more. The increase in carbopol 940 amount caused increase of viscosity. Viscosity affects the release of drug from the gel, while viscosity increases the rate of drug release decreases.

F_4 and F_5 were syringeable through 21 G needle. For drug delivery into the periodontal pocket, injectable systems are useful.[36]

The seed hull of *Q. brantii* was reported to have antibacterial activity on some gram negative bacteria.[7] Antibacterial activity against periodontal pathogens was exhibited by polyphenols present in other plant extracts.[3] In a study, antibacterial activity of galls of *Quercus infectoria* against oral bacteria was evaluated. In the galls of *Q. infectoria*, polyphenols may be the active compounds responsible for the antibacterial activity. The antimicrobial activity depends on total phenolic content in the plant extracts.[39] The data obtained in the present study revealed antibacterial activity of formulation F_5 against *P. gingivalis*. This

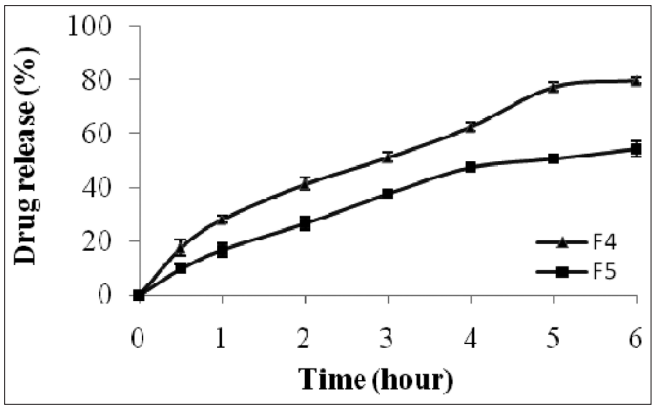

Figure 1: Percentage of cumulative drug release of formulations F4 and F5 in Franz diffusion cell through a cellulose acetate membrane, at 37°C, during 6 h

activity is attributed to polyphenols.

The ideal formulation for the treatment of periodontitis should exhibit high value of mucoadhesion and retention within the pocket for the desired period of time, show controlled release of drug and be easily delivered into the periodontal pocket preferably using a syringe.[43] Drug release from F_5 was slower and provides an extended release profile and also exhibited more mucoadhesion.

CONCLUSION

The seed hull (Jaft) of *Q. brantii* contains considerably polyphenols. Polyphenols can be used in periodontal pocket therapy due to antioxidant and antibacterial activity. Based on *in vitro* drug release and mucoadhesion studies, F_5 was selected as the best formulation. F_5 containing 1% carbopol 940 and 3% NaCMC shows satisfactory mucoadhesion property and optimum release profile. Increasing polymer concentration in the gel base can be used as a method for controlling drug release rate and for increasing mucoadhesive strength, contact time with the mucosa and residence time in periodontal pocket. F_5 produced significant growth inhibition zone against *P. gingivalis*. It is concluded that F_5 could be used as antiseptic in the treatment of periodontitis. F_5 is now the subject of clinical investigation.

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