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Formulation and in-vitro characterization of herbal licorice gel for periodontal therapy

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ABSTRACT:

Periodontal diseases are inflammations of the periodontium caused by certain microorganisms, leading to progressive destruction of the periodontal ligament and alveolar bone. Conventional medications have limitations such as extrinsic tooth discoloration, altered taste sensation, and the development of bacterial resistance. Licorice is an herb with glycyrrhetinic acid (GA) as its main active component. GA has antimicrobial, anti-inflammatory and antioxidant activity. There is limited evidence for the preparation and in vitro evaluation of licorice extract as a topical drug in periodontology. The aim of this study was the formulation and in vitro characterization of a controlled-release gel containing licorice extract that can be used as a topical agent in periodontal therapy. An in vitro study was conducted in which an ethanolic extract of licorice was prepared. The local drug delivery gel was formulated with the specified minimum inhibitory concentration of Glycyrrhiza glabra using Carbopol 940 polymer. In vitro characterization of the formulated gel was done in terms of physical appearance, determination of pH, squirtability, drug content, gelation temperature and time, viscosity, mucoadhesive force, in vitro drug release, thermodynamic stability and X-ray diffraction analysis. The licorice gel formulation was thermodynamically stable, mucoadhesive, and sprayable. It also released the drug slowly for up to 48 hours. The incorporation of licorice extract into a polymeric gel base exhibits good physicochemical properties, adequate mucoadhesiveness, and controlled drug release. Thus, it is a potential herbal extract for local drug delivery that can be used to improve the therapeutic results of scaling and root planning.

KEYWORDS: Periodontal disease; Local Drug Delivery; licorice; herbal extracts.

1. INTRODUCTION

Periodontal diseases are inflammations of the periodontium caused by specific microorganisms, leading to progressive destruction of the periodontal ligament and alveolar bone [1]. The mechanism of periodontitis development includes two main causes. One is the accumulation of periodontopathogenic bacteria in the gingival pocket [2, 3]. The other factor is the host response to the periodontal pathogenic bacteria [4].

Treatment of periodontal disease consists of plaque and calculus removal by scaling and root planning and good oral hygiene. Due to the bacterial etiology and inflammatory pathogenesis of periodontitis, the additional use of local or systemic antimicrobial agents and/or host response modulating agents has been proposed [5]. Locally applied therapy has received considerable attention due to the presence of a site-specific pattern of destruction in periodontal infections. It also provides effective local drug concentrations in the periodontal pocket and avoids potential side effects of systemic antimicrobial agents [5].

Controlled drug delivery agents are formulated to maintain the concentration of a drug for a prolonged time [6, 7]. To increase the substantivity of an agent at the site of action, controlled drug delivery requires a carrier that can deliver antimicrobial agent in a controlled manner and enhance its substantivity. Carbopols are utilized as mucoadhesive polymers. The incorporation of which results in increase of mucosal contact time

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and improvement of substantivity of the formulation [8]. The formulation is used in various biomedical preparations due to its known properties such as biocompatibility, mucoadhesiveness, biodegradability, antimicrobial and wound healing. Controlled delivery devices containing several antimicrobials agents have been examined in the treatment of periodontal disease [9]. Conventional synthetic agents have some potential limitations like extrinsic teeth stains, altered taste sensation, and development of bacterial resistance that hinder its long-term usage [10]. Herbal extracts are gaining attention since they contain phytochemicals which are naturally occurring ingredients that can achieve the desired antimicrobial and anti-inflammatory effects [11].

An example of such herb, with significant therapeutic value is "licorice (*Glycyrrhiza glabra*)". Licorice, is inherent to Mediterranean regions and few parts of Asia. The main active constituent of licorice is Glycyrrhetinic acid (GA), which is obtained from the extract of licorice root. GA shows anti-inflammatory effects due to the similarity in its chemical structure with glucocorticoids by initiation of glucocorticoid receptor signaling and also by inhibiting the classical complement pathway [12]. Licorice flavonoid components include chalcones, flavones, and isoflavones, which show antimicrobial [13], antiviral [14], anti-inflammatory [15], antidiabetic, antitumor, immunoregulatory [16], hepatoprotective [17] and neuro-protective activities [18]. The advantageous properties of licorice can be ascribed to several mechanisms. In-vitro studies have established that licorice prevents cyclooxygenase activity and prostaglandin synthesis as well as indirect inhibition of platelet aggregation and all components in the inflammatory cascade [15]. Licorice components possess important antioxidant properties. At the area of inflammation, licorice prevents the neutrophils from producing reactive oxygen species [19].

The biologically active compounds found in licorice extracts have possible effects on both oral pathogens and the host response involved in oral conditions like periodontitis, candidiasis, dental caries, and aphthous ulcers [20]. The capacity of licorice to minimize development of dental plaque adds to its role in management of periodontitis. Bioactive phytoconstituents of *G. glabra* inhibit the growth of periodontopathogens and reduce the inflammatory markers at the site of infection [21]. It also ceases osteoclastic activity that contributes to alveolar bone destruction in periodontitis and promote the synthesis of osteoblasts for new bone formation [21].

However, till date and to the best of our knowledge, the evidence available for any preparation and in vitro evaluation of licorice extract as a local drug delivery agent in the field of periodontology is limited. Therefore, the aim of the present study was to formulate and perform in-vitro characterization of a controlled drug releasing gel containing licorice extract which can be used as a local drug delivery agent in periodontal therapy.

2. RESULTS

The aim of the present in vitro study was to develop and formulate a licorice-based gel for local drug delivery in periodontal disease and to perform in vitro characterization of the gel for local drug delivery. In the present study, an ethanolic extract of licorice was prepared by maceration. To evaluate the in vitro properties, a gel for local drug administration was prepared using a polymer.

2.1. Preparation of licorice root extract:

Evaporation and solvent removal of ethanolic extracts of M. oliefera yielded a dark brown semisolid concentrated extract (Figure 1).

Figure 1: Preparation of licorice extract (a) Weighing licorice root powder (b) 70% ethanol (c) Licorice root powder mixed with ethanol (d) After 72-hour (e) Filtration using Whatman's filter paper (f) Clear filtrate obtained (g) Drying of the filtrate in hot air oven to obtain the extract (h) Licorice extract obtained

2.2 Preparation of Drug loaded Gel:

The preparation of blank gel using Carbopol 940 and distilled water produced a white semisolid gel base with good physicochemical properties. Hence, the same concentration of polymer was used as the gel base for the drug loaded gel.

A concentration of 0.1 mg/ml of prepared ethanol extract of licorice was used for the formulation of local drug delivery gel which is above the Minimum Inhibitory Concentration (MIC) against periodontal

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pathogens (*Aggregatibacter actinomycetemcomitans, Porphyromonas ginigvalis, Eikenella corrodens, Capnocytophaga sp*.).
The resultant gel formed was homogenous, semi solid and light brown in color (Figure 2, 3).

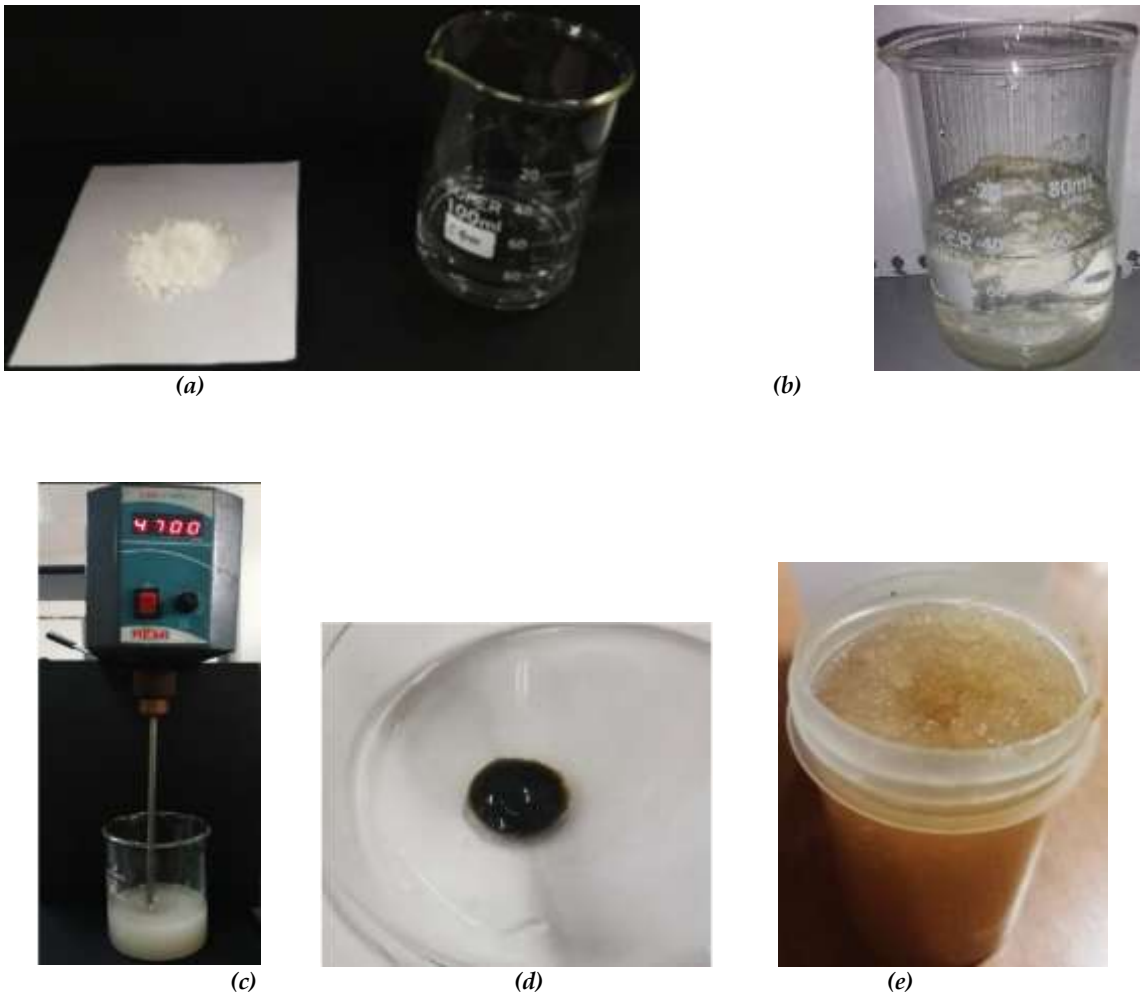

Figure 2: Preparation of blank gel and drug loaded gel (a) Carbopol 940 (b) Carbopol 940 added to distilled water (c) Mechanical stirrer (d) 1g licorice extract (e) Drug loaded gel

Figure 3: Blank gel (left) and drug loaded gel (right)

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Table 1: Physical appearance of gel formulation

	Licorice Gel
Clarity	Opaque
Colour	Light brown
Homogeneity	Homogenous
Consistency	Semi solid
Presence of particles	Yes

2.3 Invitro characterization of prepared gel:

2.3.1 Physical appearance: The obtained gel was light brown in colour, homogenous, opaque and semi-solid in consistency (Table 1, Figure 3).

2.3.2 pH determination in the gel formulation - pH meter was calibrated with distilled water at pH 7. The readings were taken after the electrode was introduced into the sample for 10 minutes at room temperature. The pH values were noted thrice and mean pH was calculated. pH of the licorice gel observed was 3.08 (Figure 4, Table 2).

Figure 4: pH determination of drug loaded gel in pH meter

Table 2: pH, drug content, gelation temperature and gelation time, and mucoadhesive strength of licorice gel formulation

Parameters	**Licorice gel**
pH	3.08
% drug content	96.25
Gelation temperature	32±0.30°C
Gelation time	33±0.1 seconds
Mucoadhesive strength	29.5 g

2.3.3 Syringeability study: The results of syringeability study indicated that the drug loaded gel was syringeable through 26 G needle (Figure 5).

Figure 5: 26 G needle

2.3.4 Drug content: Mean % drug content in the formulated gel was 96.25 (Table 2).

2.3.5 Gelation temperature and gelation time: The gelation temperature of the formulation was observed as 32±0.30°C and gelation time ranged from 33±0.1 seconds respectively (Table 2).

2.3.6 Viscosity determination: The values of viscosity determined using Brookfield viscometer are as follows (Table 3, Figure 6). The viscosity decreased as the rpm was increased from 12 to 100 rpm. At 100 rpm, the viscosity was noted ass 119.4 cps.

Figure 6: Brookefield viscometer

Table 2: Viscosity determination

Speed (rpm)	Drug loaded gel (cps)
12	381.3
20	282
30	179.6
50	170
100	119.4

2.3.7 Mucoadhesive strength: The mucoadhesive strength for the drug-loaded gel was found to be 29.5 g (Table 2). Force of adhesion and bond strength was determined as 0.289 N and 0.096 Nm^{-2}, respectively. The formulation exhibited high mucoadhesive strength, and this property could result in the retention of the drug in the periodontal pocket for the desired period of time.

2.3.8 In-vitro drug release study: In-vitro release studies showed that the licorice extract was released upto 2 days in which about 60-70% was released within 5-6 hours (Figure 7).

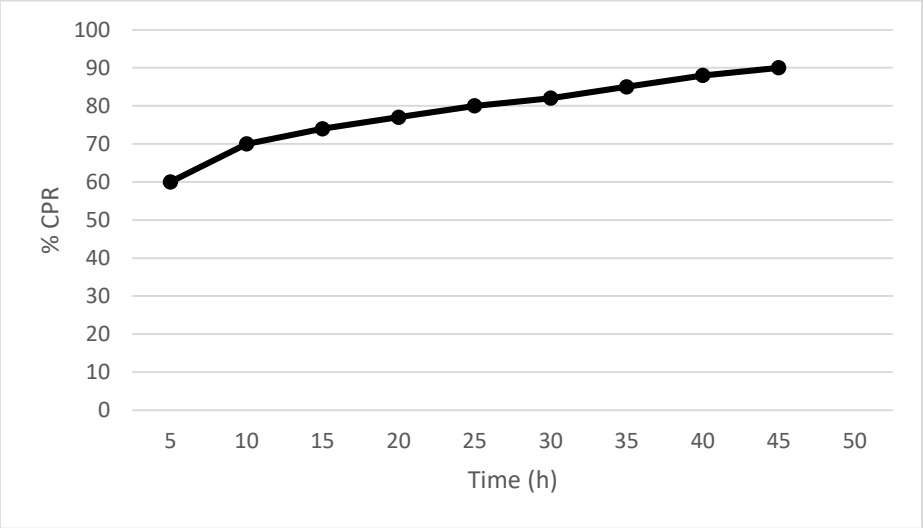


Figure 7: Cumulative percentage release of licorice extract from the gel formulation

2.3.9 Thermodynamic stability studies: In centrifugal test, freeze & thaw test and heating & cooling test, the drug loaded gel exhibited good physicochemical properties and showed stability in the experimental conditions (Table 4, Figure 8).

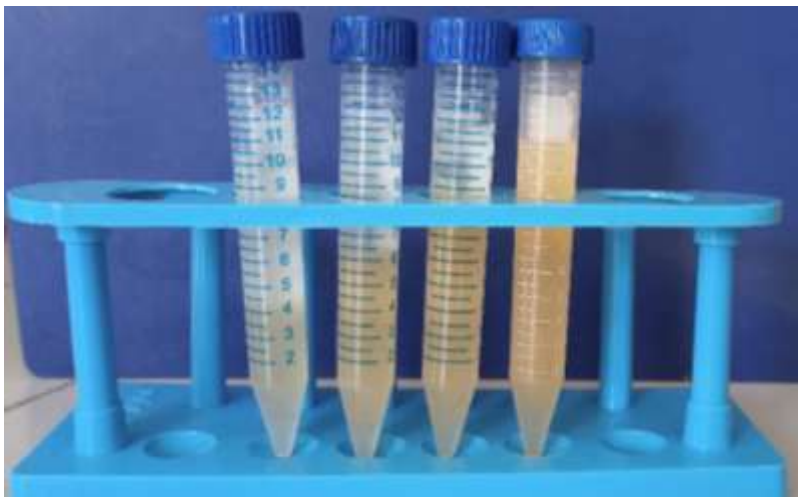


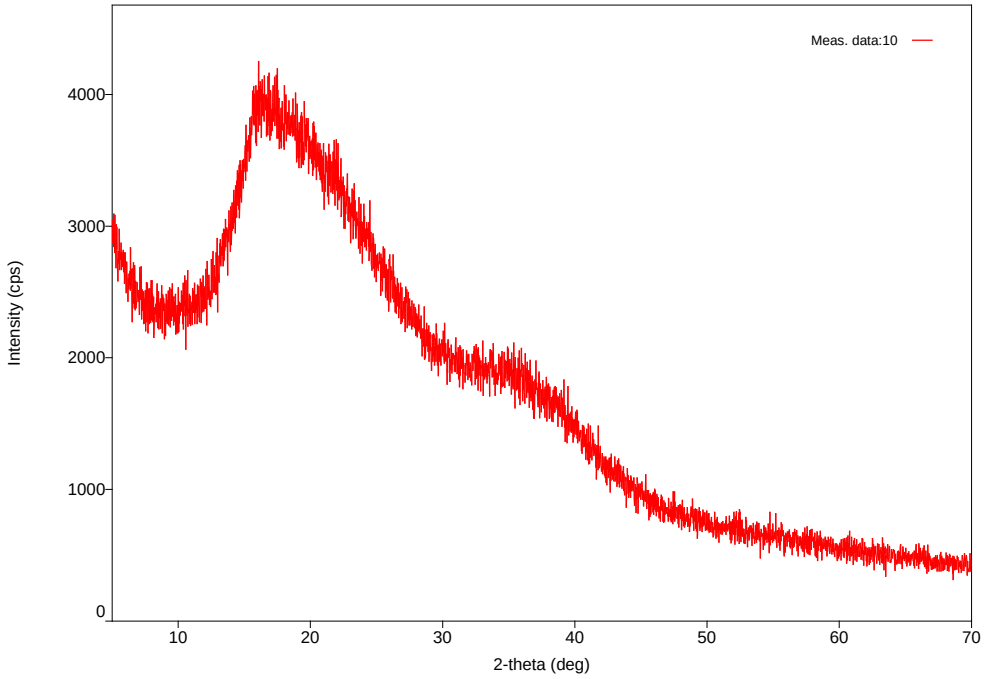
Figure 8: Centrifugal test at 15 mins

Table 4: Thermodynamic stability tests

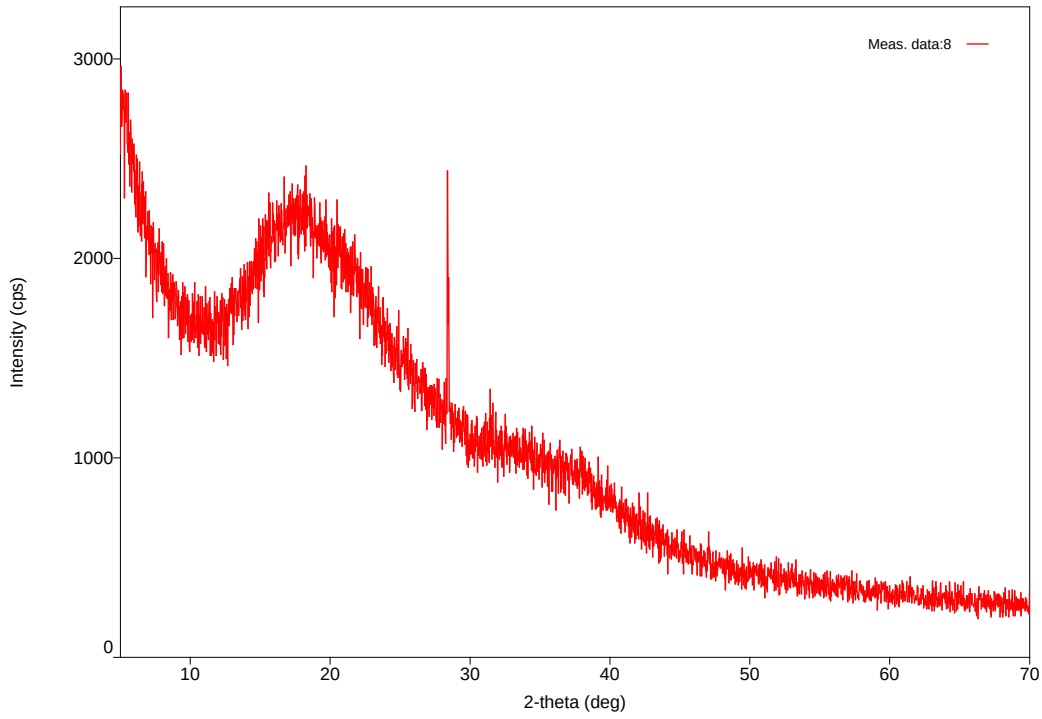
Tests	**Licorice gel**
Centrifugal test	Stable
Heating and cooling cycle	Stable
Freeze and thaw test	Stable

2.4 Analysis of X-Ray Diffraction (XRD):

The XRD spectra of carbopol exhibited peaks at θ values of 16.76° (d=5.28 Å), 37.2° (d=2.42 Å) and 39.2° (d=2.29 Å) and designated a semi crystalline nature as the peak was not sharp enough (Figure 9a). When compared the peak of the gel formulation with carbopol and the extract, the peaks observed at 18.38° (d=4.82 Å), 28.4° (d= 3.13 Å) and 37.5° (d= 2.39 Å) with two prominent peaks of carbopol (θ=18.38°) and the extract (θ=28.4°) (Figure 9b).



(a)



(b)

Figure 9: (a) XRD spectra of carbopol (b) XRD spectra of carbopol + licorice extract

The semi crystalline nature of the carbopol remains intact in the gel formulation signifying no intense interaction between the polymer and extract indicating stability in the crystalline structure of the polymer and drug in the combined form. The minor shift in the peaks of the polymer and extract could have been because of alterations in the interpolymeric density causing changes in the crystallinity.

3. DISCUSSION

Minimum inhibitory concentration (MIC) is a term used to explain the antimicrobial activity of a compound, which is the lowest concentration of the compound that can inhibit the growth of a microorganism [22]. Earlier studies have been done to evaluate the antimicrobial effect of licorice on certain predominant periodontal pathogens [23]. The results showed that the extract of licorice roots exhibited antimicrobial effect.

The MICs of glycyrrhetinic acid were 8 mg/L for A. actinomycetemcomitans and Capnocytophaga species, 16 mg/L for E. corrodens, and the MBC was 16 mg/L for all species [24]. The licorice root extract has also shown antimicrobial activity against P. gingivalis with MIC and MBC of 62.5µg/ml and 25 µg/ml respectively [25].

An ideal formulation for periodontal therapy should contain antimicrobial effects and be easily applied into the periodontal pocket [26]. Therefore, the first objective of this study was to formulate a gel containing minimum inhibitory concentration of licorice root extract against periodontal pathogens, as a local drug delivery agent in periodontal therapy.

In this study, Carbopol 940 was used as the gel base. In order to provide adequate mucoadhesiveness, carbopols expand in water to 1000 times of their original size to form a large adhesive area with mucin [27]. Other polymers form superficial bio-adhesion bonds with mucin, but carbopols form secondary bioadhesion bonds [8]. An important property of a Local Drug Delivery gel should be adequate mucoadhesive strength for adherence to the mucosa of the periodontal pocket. Addition of Carbopol 940 enhances the viscosity of the formulation and further reduces the release rate. Carbopol 940 has good swelling and cross-linking properties. The gel becomes thicker with swelling of the polymer, limiting water penetration, resulting in controlled drug release [8]. Carbopol shows excellent bio-adhesion and hence, it was used as the drug carrier in the present study.

The final objective of the study was to assess the formulated licorice extract gel with in-vitro characterization. The obtained gel was light brown in color, homogenous, opaque and semi-solid in consistency. The prepared formulation had good flexibility and a smooth, uniform surface. The mean pH of periodontal pockets was found to be 7.09 ± 0.07 in chronic periodontitis with no significant variation related to pocket depth [28]. In this study, the mean pH of drug loaded gel was found to be 3.08 which is known to cause no harmful effect to the patient. Shivaprasad et al. [29] found no adverse effect in any of the patients treated with licorice gel in their study.

The thermodynamic stability tests performed included the centrifugal test, the heating and cooling test, and the freezing and thawing test. The drug-containing gel showed good physicochemical properties and stability under the experimental conditions. The injectable application is most useful for drug delivery into the periodontal pocket. The formulated licorice extract gel could be injected with a 26-gauge needle. Viscosity affects the release of drug from the gel, with viscosity increasing as the release rate decreases. The licorice extract gel exhibited satisfactory viscosity, which can be attributed to the polymer used in the formulation.

In vitro drug release studies conducted in phosphate buffer at pH 6.8 showed a biphasic release pattern that included an initial erratic release for the first 5-6 hours, after which a slow and sustained release was observed for up to 2 days.

Licorice extract and its biologically active compounds such as glabridin, glycyrrhizin, licoricidin, licochalcone A, licorisoflavan A, have beneficial effects in periodontal disease, such as anti-adherence, anti-inflammatory, and anti-microbial properties [30-34]. Since periodontitis is a multifactorial disease, bioactive agents with dual effects such as licorice phytochemicals, can target both the host immune response and microbial pathogens. Phytochemicals are organically distinct from conventional antibiotics as microorganisms do not develop resistance against, offering a therapeutic advantage as an antimicrobial.

Previous in vitro research has shown that licorice and its bioactive constituents can be used for the treatment of periodontal disease [20]. Therefore, licorice extracts added to oral hygiene products such as toothpaste, mouthwash, gel and chewing gum need to be further investigated to confirm their beneficial effects. Local application of such bioactive agents can be more apt. For example, application of a licorice-containing gel into damaged periodontal sites, could act on periodontal pathogens and the host response by allowing the slow

and gradual release of the active ingredients. However, there is scarce evidence on the formulation and characteristics of a licorice-based local drug delivery gel for its clinical application in periodontal therapy. One limitation of this study is being an in-vitro study and only one polymer was used. Future research on the different concentrations of bioadhesive polymers in the gel base can be carried out to identify the best combination of polymers for the gel formulation to achieve ideal mucoadhesive property. Another limitation was that the antimicrobial efficacy of the gel against periodontal pathogens was not evaluated. As a result, further antimicrobial studies must be conducted to reveal the efficacy of the licorice gel on broad range of microorganisms. Future in vivo clinical studies should be done to assess the clinical efficacy of the formulated gel as a local drug delivery agent in periodontal therapy.

4. CONCLUSION

The results of the present study showed that the licorice gel formulation was thermodynamically stable, mucoadhesive and injectable. Moreover, the active ingredient was slowly released for up to 48 hours. The incorporation of the extract into the polymeric gel base exhibits good physicochemical properties, adequate mucoadhesiveness, and controlled drug release. Therefore, licorice root extract is a potential herbal extract for local drug delivery that can be used in the elimination of deep periodontal pocket infections, which could improve the therapeutic results of scaling and root planning.

5. MATERIALS AND METHODS

The study was an in-vitro study and ethical clearance was not applicable. The licorice extract was prepared and the local drug delivery gel was formulated with the established minimum inhibitory concentration of licorice extract, against periodontal pathogens [24, 25] using Carbopol 940 as the polymer. In vitro characterization of the formulated gel was done.
Licorice root powder was procured from Jain Lifesciences Pvt Ltd, Hyderabad. 70% ethanol was procured from Chemical House, Bengaluru, Karnataka. Carbopol 940 was obtained commercially from SD Fine Chemicals, India.

5.1 Preparation of licorice root extract:

The dried powdered roots (100 g) were macerated with 70% ethanol (400 mL) (1:4 w/v) with occasional shaking for 72 hours at room temperature (28 ± 2 ˚C). Whatman no. 1 filter paper was used to filter the extract and the marc was re-extracted by the same process until the extraction was exhausted. The extract was then evaporated to dryness at 90 ˚C in a hot air oven.

5.2 Preparation of Blank Gel:

O.5 g Carbopol 940 was dissolved in 10 ml of water by stirring constantly with a mechanical stirrer at 3000 r/min for 30 mins.

5.3 Preparation of Drug loaded Gel:

4g Carbopol 940 was dissolved in 80 mL of water by stirring constantly with a mechanical stirrer at 3000 r/min for 30 mins. 8 mg of licorice extract was added to it and mixed well together with the mechanical stirrer for 30 min at ~5000 r/min. The mixing was continued for 1 hour until a clear gel was obtained.

5.4 Invitro characterization of prepared gel:

5.4.1 Physical appearance - The formulated gel was visually assessed for color, clarity, consistency, homogeneity, and presence of particles [27]. Consistency of the formulations were investigated by pressing a small amount of gel in between the index finger and thumb [35]

5.4.2 Determination of pH in gel formulations**:** Calibration of the pH meter was done with distilled water at a neutral pH of 7 before each use. The readings were taken after the electrode was introduced into the sample at room temperature for 10 min. The pH values were noted thrice and mean pH was calculated [36].

5.4.3 Syringeability study**:** Syringeability of gel formulations was evaluated by injecting the gel formulation through 26 G needle [37].

5.4.4 Drug content: 1 ml of formulated gel was transferred to 50 ml of pH 6.8 phosphate buffer in 100 ml of volumetric flask with constant shaking to give a clear solution. Buffer was used to adjust the final volume to 100 ml. After appropriate dilutions, the concentration was calculated at 235 nm (wavelength of maximum absorption) through UV spectrophotometer (Shimadzu Pharmspec UV 1700, Japan) [38].

5.4.5 Gelation temperature and gelation time**:** Gelation temperature was measured by using the procedure described by Baloglu et al. [39] 5 ml of the preparation was transferred to a 20 ml transparent vial having a magnetic bar which was placed in a water bath (at 4°C). The temperature was gradually increased with continuous stirring at 200 rpm. Gelation temperature was taken as the displayed temperature on the immersed thermometer when the magnetic bar stopped moving due to gelation. For the gelling time, the time taken for solidification was noted after 5 ml of the gel was transferred to a 20 ml test tube and placed in a water bath maintained at 37 °C [38].

5.4.6 Determination of viscosity**:** Brookfield viscometer [DV-II+ Viscometer (Brookfield Engineering Labs Inc., USA)] was used to determine the viscosity of the formulation. The polymeric dispersion was transferred into a test tube after mixing, and the viscosity was assessed for the formulation using spindle no. 4 at 100 r/min [40].

5.4.7 Ex-vivo mucoadhesive strength**:** Mucoadhesive strength was determined in freshly collected porcine buccal mucosa using a fabricated mucoadhesive strength test apparatus (Figure 10, 11) [41].

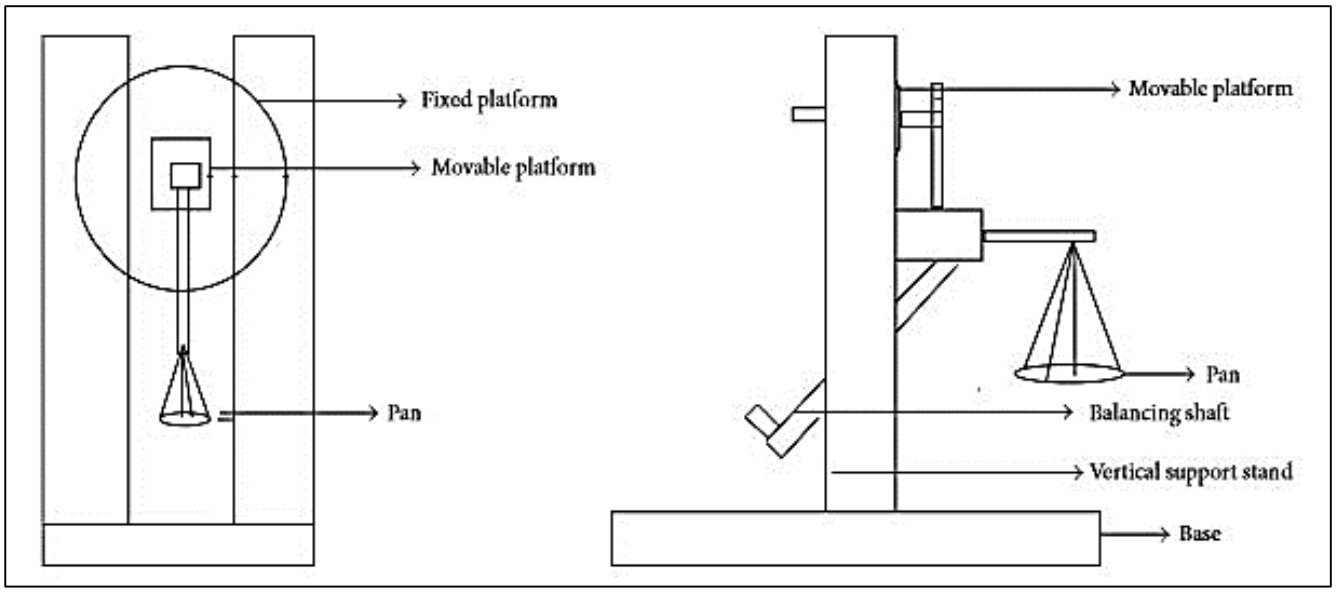


Figure 10: Diagrammatic representation of mucoadhesive strength test apparatus

Figure 11: Porcine cheek mucosa

The test apparatus had a base metal that held a vertical support stand. The stand consisted of two platforms- one is movable and the other is a fixed platform. The balancing of the movable platform with the apparatus was done with a shaft, that was balanced to keep the equipment steady. To hold the weight, a pan was put on the apparatus and further to measure the bio-adhesive strength. Buccal mucosa of porcine origin was procured from a local butcher and it was used within two hours of sacrificing the animal. The underlying adipose and loose tissue were removed from the mucosal membrane and washed in distilled water followed by phosphate buffer (pH 6.8) at 37◦ C. A mucosal layer of 3 cm^2 was fixed on the immovable platform using cyanoacrylate gum after the preparation of the tissue.
Similarly, the sample film was fixed using cyanoacrylate gum to the movable platform. For swelling and initial hydration, the film surface that was exposed was hydrated with 15 μL of phosphate buffer and left for 30 seconds. The movable platform was brought into contact with the mucosal surface by making it shift towards the fixed platform horizontally. To ensure proper film attachment to the mucosal surface, a preload of 20g was kept on the movable platform for 3 minutes as the initial pressure. Addition of weights on to the attached pan with the movable platform was then carried out proportionately at a definite time interval. The total weight needed for initiation of complete film detachment was recorded, and the various mucoadhesive strength characteristics were calculated as follows:

Force of adhesion= (Bio-adhesive strength × 'g')/ 1000
Bond strength= Force of adhesion/ Film surface area

Bio-adhesive strength is the mass in grams that is needed to separate the film from the mucosal surface. 'g' is referred to as the acceleration due to gravity. Its value is 9.81 m/s^2. The energy required for detachment of the two systems is defined as Bond strength.

5.4.8 In-vitro drug release study**:** In-vitro drug release study of the formulated gel was done by using the dialysis method [42]. Dialysis membrane (Himedia labs) was soaked overnight in 1:1 mixture of phosphate buffer at pH 6.8 and glycerol. Each bag was loaded with 2 ml of the gel, hermetically sealed and dialyzed against 200 ml phosphate buffer in a 500 ml beaker, maintaining a temperature of 37±0.5°C, kept on a thermostatically controlled magnetic stirrer and stirring at 100 rpm. The samples were withdrawn at predetermined time intervals of 0, 0.5, 1, 2, 3, 4, 5, 6, 24 and 48 h, and to maintain sink condition it was replaced with equivalent quantity of buffer. The amount of drug released was calculated using UV spectroscopy as discussed earlier.

5.4.9 Thermodynamic stability studies: Thermodynamic stability tests were carried out on the formulation by the following methods [43]:

Centrifugal test - A centrifugal device (Centrifuge 5430) was used to evaluate the stability of the formulation against centrifugal force. Gel formulation was put into tubes and then centrifuged at 2000 r/min for 15 min [37].

Heating–cooling cycle - The formulation was kept at 45 ˚C and 0 ˚C alternately for 48h and noticed for physical changes [44].

Freeze and thaw test - The prepared formulation was exposed for three consecutive cycles to -20 ˚C and 20 ˚C for 24h each and noticed for physical changes [44].

5.4.10 X-Ray Diffraction analysis:

It is a specific study of the structure of crystalline phases which is based on the potential of crystals to diffract X-rays in a characteristic method. Constructive or destructive scattered radiation occur because of the periodic nature of a crystalline material, resulting in characteristic diffraction phenomena which is studied to evaluate the crystal structure of materials [45].

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Conflict of interest statement: The authors declared no conflict of interest.

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