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Formulation and Characterization of Thymoquinone Bioadhesive Gel for Treatment of Chronic Gum Inflammation

Eman Alaaeldin[a*], Mohamed Diaa Gomaa[b], Nael Kamel Eltewacy[c], Amna Ali[d], Ahmed Abdallah Khalil[e]

[a,b,c,d]*Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia 19623, Egypt*

[e]*Lecturer of Oral Medicine, Oral Diagnosis and Periodontology, Faculty of Dentistry, Minia University,Minia 19623, Egypt*

[a]*Email: eman_alaa_eldin@yahoo.com*

Abstract

The aim of study is to formulate Thymoquinone gel and investigate its effect in chronic periodontitis in terms of clinical periodontal parameters, anti-oxidant capacity and the levels of IL-1β in gingival crevicular fluid. different gelling agents (carbopol, hydroxyl propyl methyl cellulose and chitosan) were used for the development of TQ gel. The optimized gel formulation was used for the clinical study. The study was conducted on 68 subjects (25-58 years old). 48 patients were clinically diagnosed with moderate to severe chronic periodontitis. Patients were divided into three groups; Group I (24 patients): received non-surgical periodontal therapy and Thymoquinone-chitosan gel (0.1 % w/w), Group II (24 patients): received only non-surgical periodontal therapy and Group III: 20 healthy subjects (control group). All patients were evaluated for clinical parameters including plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL). The levels of IL-1β and total anti-oxidant capacity were recorded in gingival crevicular fluid at baseline (prior to treatment) and at weeks 4 and 12 after treatment. **The results** showed that combination of non-surgical periodontal therapy and Thmoquinone gel (group I) exhibited statistically significant improvement in biochemical parameters compared to non-surgical periodontal therapy alone (group II).

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* Corresponding author.

In conclusion we can say that the adjunctive use of thymoquinone gel with non-surgical periodontal therapy improves the biochemical parameters accompanied with chronic periodontitits significantly after 4 weeks only.

Key words: periodontitis; TQ; bioadhesive gel; IL-1β; clinical parameters.

1. Introduction

Nigella sativa or black seed is an annual plant of the Ranunculaceae family. Nigella sativa seeds contain a variety of well-known active compounds with many pharmacological effects[1]. Thymoquinone (TQ) is the main bioactive constituent of Nigella sativa oil. It exhibits numerous pharmacological properties, including, ant-ibacterial, anti-inflammatory, anti-histaminic, anti-oxidant and hypoglycemic actions [2, 3]. Periodontitis is a chronic inflammatory disease, characterized by gingival bleeding, periodontal pocket formation, connective tissue destruction and alveolar bone resorption which may result in tooth loss. Although oral bacteria, and their toxins, enzymes and metabolites, are considered to be the main causative factors for the initiation of inflammatory processes, the host inflammatory response is primarily responsible for the progression of the disease and for most of the connective tissues destruction [4, 5]. Inflammatory mediators are important to the pathogenesis of periodontal diseases and may be used as diagnostic markers. Interleukin (IL)-1 is present in two active forms, IL-1α and IL-1β. Both are potent Pro-inflammatory molecules and are the main components of osteoclast activating factor [6]. Reactive oxygen species (ROS) have been implicated in the pathogenesis of many inflammatory diseases, including rheumatoid arthritis[7], chronic obstructive pulmonary disease [8], atherosclerosis[9] and periodontal disease[10]. The total antioxidant capacity (TAOC) has been widely used to investigate OS in periodontal disease. It is an integrated parameter that reflects the cumulative activity of nonenzymatic antioxidants present in the plasma and body fluids[11]. Many treatment modalities are available to achieve the goal of periodontal therapy. This includes nonsurgical periodontal therapy, such as scaling and root planing (SRP) alone or SRP plus systemic or local antimicrobial/anti-inflammatory agents, and surgical periodontal therapy[12]. Bioadhesive gels for buccal application should maintain suitable mechanical and rheological properties, including good spreadability, suitable PH for buucal environment, appropriate hardness, pseudoplastic or plastic flow with thixotrophy, and prolonged residence time [13]. These properties may enhance the patient compliance and adherence to administration instructions. Moreover, the leaky epithelium of the buccal region would enhance the absorption of applied drugs. Avoidance of enzymatic degradation and maximized absorption as aresult of the copious blood supply are valuable advantages of buccal route of drug delivery. Conversely, the application of common dosage forms such as creams, ointments, solutions, and onto the oral mucosa is counteracted by salivation, swallowing and tongue movement. Hence, new applicable formulations with controlled release should be considered. The mucoadhesive dosage forms prepared using certain water-soluble polymers maintain adhesiveness on hydration and e can be utilized for targeting the delivery drugs to certain region of the body. The ultimate performance of buccal preparations and their acceptance by patients are dependent on their suitable rheological, pseudoplastic or plastic flow with thixotrophy, ease of application, good spreadability, and extended residence time in the oral cavity. In the present study, bioadhesive gel-forming agents with suitable gelation temperature like Carbopol and Poloxamer were used for excellent thick gel barrier formation characteristics [14]. The prepared TQ gel was evaluated for its effect on the clinical periodontal parameters as well as the level of IL-1β and total anti-oxidant

capacity levels in gingival crevicular fluid of chronic periodontitis patients.

2. Materials and methods

2.1. Materials

Thymoquinone (2-isopropyl-5-methylbenzo-1, 4-quinone) > 99 % was purchased from Sigma-Aldrich (St. Louis, MO, USA). Carbopol 934 was purchased from Lobo Chemie Pvt. Ltd., India. Poloxamer ١٨٨ was purchased from Himedia Lab Pvt .Ltd., India. Deionized water was supplied by the microbiological laboratory for water and food analysis (Minia, Egypt). All other chemicals used in the study were of analytical grade.

2.2. Methods

2.2.1. Preparation of Thymoquinone bioadhesive gel

Carbopol-poloxamer gel of 1% thymoquinone was prepared as follows: minimum amount of water was added to Poloxamer 188 (2%) at 5°C with gentle stirring[15]. Subsequently, TQ was added with minimal amount of ethanol to the poloxamer solution. An aqueous suspension of Carbopol 934 (5%) was stirred with in a separate beaker and added to the prepared solution of Poloxamer-TQ and stirred for 1h. The preparation was then adjusted to 25 ml with distilled.

2.2.2. Characterization of TQ bioadhesive gel

2.2.2.1. Measurement of gelation temperature

Two grams of TQ gel was placed in a 100 ml beaker above a low-temperature thermostat water bath. Prepared gel was continuously stirred at 50 rpm at with increasing the temperature by the rate of 5°C/5 min [16]. The temperature at which the magnet stopped moving as a result of gelation was recorded as the gelation temperature.

2.2.2.2. Determination of bioadhesive force

The bioadhesive force of the prepared TQ gel was determined using a modified balance[17]. Minimal weights required for detaching the mucosal side of goat buccal tissue secured on two vials and enclosed by the prepared gel was recoreded as the bioadhesive force.

2.2.2.3. Rheological study

Clarity of the prepared gel formulation was evaluated through visual inspection against black and white backgrounds. pH of the prepared formulation was measured using pH meter. Experiments were carried out in triplicates and the pH result were the average of the three determinations. Moreover, the rheological profiles of the prepared gel formulation were studied and recorded using Anton paar rheometer (RHEOPLUS/32 V3.40 software). Alternating rotational speed of the rheometer spindle (1 to 50 rpm) was applied to perform different shear rates. The viscosity at a given shear rate was determined at 37±1 ^{O}C and plotted against rate of shear.

2.2.3. Ex- vivo permeation of drug through the buccal mucosa

Ex- vivo permeation studies were performed using Freshly excised goat buccal tissue within 2 h of removal [18]. Surgical scissors were used for removing the underlying tissues keeping the basal membrane intact. The prepared buccal mucosa was then stored at 4°C for 24 h in phosphate buffered saline pH 6.8. Modified Franz diffusion cells were used for the permeation study . Specially designed diffusion tubes with goat buccal mucosa at one end were used by placing Two g gel inside. This was immersed in a beaker containing 20mL of Phosphate buffer (pH 6.8) and was shaken in thermostatically controlled magnetic stirrer at 37±1°C. Cumulative amount permeated of TQ was determined spectrophotometry at 256 nm. The release data of TQ gel was fitted to zero-order, first-order, and Higuchi equations to determine the kinetic model of drug release.

2.2.4. Clinical study

To gain more insight into the effect of the combination of application of TQ bioadhesive gel and non-surgical periodontal therapy on the improving the periodontal clinical parameters, sixty eight subjects of both sexes with age ranged 25-58 years old were divided into three groups: Group I: healthy subjects (control group), Group II: 24 patients with moderate to severe chronic periodontitis received only non-surgical periodontal therapy including three sessions of full mouth supra and sub gingival scaling and root planning (SRP) using hand instruments and ultrasonic scalers , Group III: 24 patients with moderate to severe chronic periodontitis received non-surgical periodontal therapy combined with TQ gel treatment where Periodontal pockets were filled with the TQ gel 0.1 % (w/w) immediately after completion of SRP using a blunt sterile needle until the gel could be observed at the gingival margin. The gel was reapplied after 48 hours and the gel applied sites were covered with a periodontal pack for 7 days. The patients were instructed to keep their oral hygiene procedures except the sites covered with dressing and after the removal of the dressing the operation site was washed with sterile saline. Subjects were selected from the out patients clinic of the Oral Medicine, Oral Diagnosis, and Periodontology Department. Faculty of Dentistry, Minia University. The complete treatment protocol was explained to all patients and their full signed consent was obtained. The study was fulfilled with the rules set by the International Conference of Harmonization Good Clinical Practice Guidelines, and the Declaration of Helsinki and the research ethics committee of the Faculty of Dentistry, Mini University.

2.1.4.1. Evaluation of clinical parameters

Plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL) were recorded at baseline (prior to treatment) and after 4 and 12 weeks of treatment in group II and III.

2.1.4.2. Determination of IL-1β and TAOC levels in gingival crevicular fluid (GCF)

Samples were collected from the GCF of the three groups at baseline, 4 weeks and 12 weeks after treatment. Samples were collected using Whatman3MM Chromatography (Wh.3MM) absorbent filter paper strips at baseline, 4 and 12 weeks. The supragingival plaque was isolated from maxillary teeth to avoid contamination with saliva. Buccal sites only were used to collect GCF samples to ensure approachability and the areas were

gently air dried. The paper strip was inserted into the gingival crevice up to 1 mm so that mild resistance was felt and keptt in the crevice for 30 seconds. The strips were individually stored in plastic Eppendorf and at - 20º C kept for further investigations. GCF from filter paper strips was diluted with the Reaction Buffer and ultra-filtered through a 10,000 MWCO filter then used directly in the assay. Samples were incubated and centrifuged at 4°C. Commercially available Enzyme linked immune-sorbent assay (ELISA) kits were utilized, and assays were applied according to the manufacturers' instructions to examine the level of IL-1β and TAOC.

3. Results

3.1. Evaluation of bioadhesive gel of TQ

3.1.2. Gelation temperature

Gellation of Poloxamer-cabopol gel of TQ occurred at 35 ºC and that was in continenece with a previous study that reported that combination of carpobol 934 with poloxamer 188 increases the gelling temperature of poloxamer to the physiological temperature [17]. Physically the prepared gel formulation was clear, homogenous, and the pH was ranging from 5 to 5.3 suggesting suitability to skin application.

3.1.2. Bioadhesive force

The buccal mucosal membranes are composed of oligosaccharide chains which can bind with polymers containing hydrophilic groups with certain bioadhesive force. Results show that TQ bioadhesive gel maintained a moderate bioadhesive force of 42 g force.

3.1.3. Rheological study

The prepared gel formulation exhibited pseudo-plastic behavior where viscosity is decreased by increasing the shear rate (Figure 1). Pseudopalstic behavior is favorable in pharmaceutical gel preparations to allow neat preparation, good spreadability, proper handling, and application on the skin.

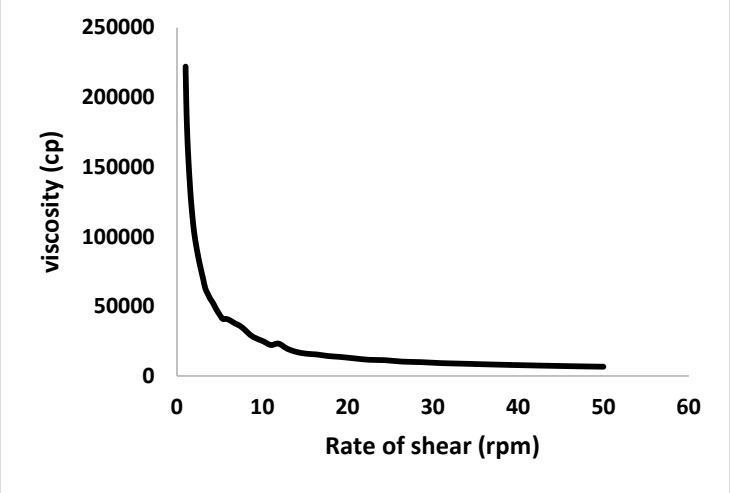

Figure 1: Shear rate-viscosity diagram of TQ- gel

3.2. Ex-vivo permeation

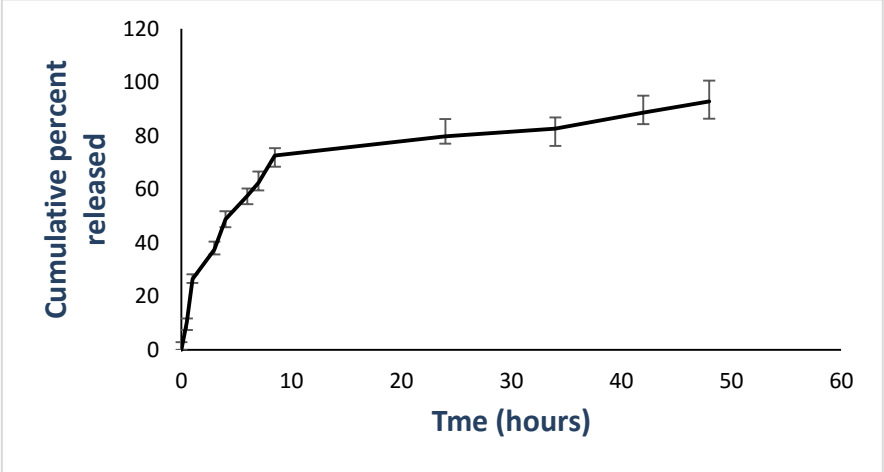

Figure 2: Cumulative percentage released of TQ gel

Table 1: kinetic evaluation of release data

Formulation	zero-order	First-order	Higuchi
TQ gel	0.9654	0.9732	0.9934

Release of TQ from the prepared gel is shown in **figure 2**. About 60 % of TQ was released through goat buccal membrane within the first 10 hours and reached 90% after 50 hours. The release through buccal mucosa exhibited a higuchi model (Table 1).

3.3. Clinical study

3.3.1. Clinical parameters

The clinical parameters including gingival index (GI), probing depth (PD) and clinical attachment level (CAL) were recorded at baseline (prior to treatment) and at weeks 4 and 12 after treatment in group I and II. Both groups showed significant improvement regarding all clinical parameters from baseline to 4 and 12 weeks after treatment. The changes in the clinical parameters of the two studied patients groups are summarized in table 2.

Table 2: percentage improvement in clinical parameters in group II and group III at different intervals.

Groups	GI		CAL		PD	
	4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks
Group II	47.96±0.2 %	71.9± 0.15 %	21.71± 0.50 %	47.7±0.35 %	29.19± 0.043 %	50.93±0.02 %
Group III	49.95±0.05 %	75.27±0.83 %	27.72±0.03 %	58.5±0.081 %	33.94±0.2 %	56.9±0.13 %

3.3.2. Biochemical evaluation evaluation of IL-1β and TAOC levels in GCF

The collected samples from the GCF of group I, group II and group III at baseline, 4 weeks and 12 weeks after treatment were examined for the level of IL-1β and TAOC using Commercially available Enzyme linked immunosorbent assay (ELISA) kits. Changes in the mean values of IL-1β at baseline and at different intervals in groups I, II and III were shown in table (3). Results show significant reduction in IL-1β after four weeks of treatment with combined clinical treatment and TQ gel (group III) compared to single clinical treatment (group II), P <0.0001. A consecutive significant reduction in levels of **TAOC** was determined in group III compared to group II, P <0.0001 (table 4).

Table 3: Independent sample t- test to compare IL-1 β, between group I, group II and group III at different intervals.

IL-1 β (pg/µl)	Group I	Group II Mean±SD	Group III Mean±SD	P value (II versus III)
Baseline	103. 2±12.5	230.49±24.73	233.99±25.11	0.6
After 4 weeks	102.42±10.4	153.26±27.31	125.54±19.21	<0.0001
After 12 weeks	102.1±11.25	106.04±9.51	103.95±10.84	0.4

Table 4: Independent sample t- test to compare TAOC, between group I, group II and group III at different point of time

TAOC (µm/l)		**Groups**		P-value (II versus III)
	Group I	Group II Mean±SD	Group III Mean±SD	
Baseline	843.33±22.52	644.22±17.02	647.37±22.06	0.5
After 4 weeks	840.43±11.5	723.91±12.62	739.54±15.88	<0.0001
After 12 weeks	838.2±20.3	762.96±12.84	773.73±14.68	0.01

4. Discussion

Pharmacological investigations of *Nigella Sativa* seed extracts reveal that Thymoquinone (the main active constituent) possess a broad spectrum of activities including antioxidant, anti-inflammatory[19, 20] and antimicrobial potential against both Gram-positive and Gram-negative bacteria[21]. The main hypothesis of this work is to estimate and maximize the potential of this cargo in the treatment of periodontitis (an inflammatory disease leads to local elevation in levels of pro-inflammatory cytokine which plays a vital role in the process of inflammation associated with the destruction of the periodontium[22, 23]. A bioadhesive TQ gel was prepared using poloxamer 188 and carbopol 934 first to sustain the rate of release of TQ so that the preparation could be

applied twice only, and second to increase the contact time of the gel with buccal mucose and counteract the flushing effect of saliva. The gelation of the prepared bioadhesive gel occurred at 35°C so gelation occurs after biological application and not at the room temperature. Addition of carbopol 934 kept the well-arranged zigzag configuration of Poloxamer 188 and allowed gelation at 35°C [17]. The gel showed an optimum bioadhesive force which was enough to keep the drug in contact with the mucosal membrane without any damage for the mucous membranes. The pH of the TQ gel kept fairly constant the range of pH (6.7–7.0) along the 6-h test period, approving Suitability of the prepared gel to the natural environment of the Buccal acvity (pH 5.8–7.1). The controlled drug diffusion via the buccal mucosa can be attributed to the marked swelling of Carbopol 934 which extensively creates a thick gel barrier. The prepared TQ gel combined with non-surgical periodontal treatment was evaluated in terms of enhancement of clinical periodontal parameters and reduction of anti-inflammatory mediators (IL-1β) [24]and elevation of anti-oxidative stress (TAOC) [25]. Results show that either single non-surgical periodontal treatment alone or combined with TQ bioadhesive gel treatment would significantly enhance the Periodontal probing depth, clinical attachment level, gingival index and plaque index compared to the control group. The enhancement in the clinical parameters was more pronounced in the groups received combined treatment (group III). The improvement of clinical parameters in both groups may be due to the non-surgical clinical therapy. Scaling and root planning approach is efficient in reducing the magnitude of calculus and bacterial biofilm adherent to the sub-gingival surface [26]. The superior improvement of the clinical parameters owing to combined treatment could be attributed to the synergistic anti-oxidant and anti-inflammatory effect of the sustained-released TQ of the bioadhesive gel. Considering the biochemical mediators, the elevated the baseline levels of IL-1β in patients with periodontitis (groups II, III) compared to the control group (group I) was in consistence with other studies [27, 28]. IL-1β concentration in GCF was significantly decreased in both groups after 4 and 12 weeks of treatment compared to the base line concentration of each individual group. Levels of TAOC have been significantly increased in the after 4 and 12 weeks of either single or combined treatment compared to baseline. Groups received combined treatment shows significant enhancement in the levels of biochemical parameters after four weeks compared to the biochemical parameters of the group with the single non-surgical clinical treatment only. However, the improvement was not significant after 12 weeks of treatment, this may be attributed to physical instability and photosensitivity of TQ [29]and, binding to plasma proteins of TQ (more than 99%) [30].

5. Conclusions

Combined treatment of periodontitis with non-surgical clinical treatment and bio-adhesive thymoqinone gel shows significant enhancement in the biochemical parameters of periodontitis after 4 weeks of treatment compared to single non-surgical periodontal therapy. Combined treatment has failed to keep that significant improvement following 12 weeks of treatment due to the physical instability of the drug. Further studies are managed by our lab. including the development of an advanced nanocarrier system to enhance the physicochemical properties and pharmacokinetics of the drug via microencapsulation.

6. Recommendation

Further studies are to be attempted to encapsulate TQ within nanocarrier dosage forms to enhance its stability

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