



ORIGINAL ARTICLE

Assessment of clinical efficacy of locally delivered 0.2% Thymoquinone gel in the treatment of periodontitis

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KEYWORDS

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Gingival index;
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Probing pocket depth;
Relative attachment level;
Thymoquinone

Abstract *Objectives:* To evaluate the potential benefits of local application of Thymoquinone gel as an adjunctive to scaling and root planing (SRP) in subjects with chronic periodontitis.

Material and methods: Twenty subjects with 40 test sites were selected according to inclusion and exclusion criteria. They were further divided into 2 groups. Group I comprised of study subjects (Thymoquinone in addition to SRP) and Group II comprised of control subjects (only SRP). Clinical parameters such as Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), Relative Attachment Level (RAL), were monitored at baseline and 6 weeks post operatively. Alkaline phosphatase (ALP) levels in gingival crevicular fluid (GCF) were evaluated at baseline and 6 weeks post operatively using microcapillaries. In addition antimicrobial efficacy of Thymoquinone was evaluated against 3 bacteria using antimicrobial strains.

Results: Statistically highly significant reduction was observed in PI, GI and PPD, rise in RAL and GCF ALP level in both the groups at 6 weeks from baseline. On comparison between Group I and Group II, former demonstrated statistically significant reduction in PPD, GCF-ALP levels and rise in RAL but statistically no significant differences were observed in PI and GI at 6 weeks. On microbiological assessment of 0.2% Thymoquinone gel, it was observed to be sensitive against *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia*.

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Conclusion: Significant changes in clinical and biochemical parameters were achieved in the current study. Hence, it is concluded that intracrevicular application of 0.2% Thymoquinone gel could be a beneficial adjunct to SRP in treating chronic periodontitis.

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1. Introduction

Periodontitis is an inflammatory and destructive, pathological condition that affects the connective tissue attachment of the teeth affecting mostly the adult population of the world. It is characterized by elevated host response against the associated gram-negative pathogens usually organized as a biofilm, destroying cells and supporting tissues, which ultimately causing tooth loss (Breivik et al., 2006; Brotto et al., 2011; Duang et al., 2011). It has been proved that an increase in oxidative stress and diminished antioxidant capacity are major factors responsible for destruction of periodontal structure (AbouSulaiman and Shehadeh, 2010). Patients pertaining to periodontitis demonstrate an oxidative stress in the oral cavity (D'Aiuto et al., 2010).

Oxidative stress results from imbalance between generation of free radicals and antioxidants, regulating the oxidative reactions by inhibiting/delaying/hampering the oxidation of substances. The 'imbalance' resulting in oxidative stress can be hampered by increased free radicals, or by reduction in the amount of anti-oxidative substances, further causing lipid peroxidation; injured DNA; or degradation of cellular proteins. Additionally oxidative stress has been related to a number of chronic inflammatory diseases (AbouSulaiman and Shehadeh, 2010).

With emerging awareness concerning the role of oxidative stress associated with periodontal disease, host-modulator therapies are being investigated which regulate the role of antioxidants in preventing the breakdown of soft and hard periodontal tissues (Toker et al., 2008; Toker et al., 2009; Tomofuji et al., 2009). Taking into consideration their anti-oxidative and anti-inflammatory properties, herbal therapy is also a special area of research for the prevention and treatment of various periodontal diseases. *Nigella sativa* is a widely used plant in traditional medicines particularly in Middle-Eastern and Asian countries for treatment of wide range of ailments, most commonly including rheumatoid pain, hypertension and asthma (Chehl et al., 2009).

Recently, it has been observed that most of the properties of *Nigella sativa* or its extracts are attributed to Thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone), which is one of the monoterpenoid hydrocarbon compounds of *Nigella sativa*'s volatile oil (Sultan et al., 2009) having anti-oxidant (Chaieb et al., 2011; Iauk et al., 2003; Mansour et al., 2001; Sultan et al., 2009; Vahabi et al., 2011) and anti-inflammatory properties (Chehl et al., 2009; El-Dakhakhny et al., 2006; Tekeoglu et al., 2007).

Considering the above merits, the present study was undertaken with an aim to access and compare the efficacy of locally delivered 0.2% Thymoquinone gel in the treatment of chronic periodontitis on the basis of various clinical and biochemical parameters.

2. Materials and methods

2.1. Study population

The present study was carried out in the Department of Periodontology and Oral Implantology, as per the Declaration of Helsinki (1964 revised in 2008), with the approval of Institutional Ethical Committee of the University, which included a total of 40 sites from 20 patients aged between 23–61 years (mean age 36.25 years, 15 males and 5 females) (Antczak-Bouckoms et al., 1990; Ramfjord et al., 1968).

The subjects were selected from the outpatient department and were divided into 2 groups. Group I served as study groups comprising of subjects in which 0.2% Thymoquinone was administered in addition to scaling and root planing, and Group II served as control group comprising of subjects in which only scaling and root planing (SRP) was done. All the participants were given detailed verbal and written description of risks and benefits of the treatment and a written consent was subsequently obtained.

2.2. Selection criteria

Both male and female subjects with at least 2 sites of periodontal pocket one in each quadrant of maxillary teeth, having a probing depth of ≥ 5 mm with radiographic evidence of bone loss were included in the study. The sites were chosen from maxillary teeth to avoid contamination with saliva (Sanikop et al., 2012).

Subjects were excluded from the study; if their systemic health precluded periodontal treatment; if they were pregnant; if they had any known allergy or hypersensitivity to any product used in the study; if they had previous periodontal and/or antibiotic therapy in the last 6 months.

2.3. Gingival crevicular fluid collection and alkaline phosphatase level estimation

The selected sites from maxillary teeth were isolated with the help of cotton rolls and were dried with a gentle stream of air. Gingival crevicular fluid (GCF) samples were collected with the help of calibrated microcapillary tubes (0–5 μ l range) placed extracrevicularly at gingival margin and were held in the same position until 5 μ l of the GCF was collected. The collected GCF was immediately transferred to a sterilized microcentrifuge (Eppendorff) tubes that contained 45 μ l of normal saline. Then the samples were subjected to alkaline phosphatase (ALP) level estimation carried out at baseline and 6 weeks postoperatively.

ALP levels were determined by using a commercially available diagnostic kit (Erba Chemicals, Mallaustr, 69–73,

D-68219, Mannheim/Germany) and the samples were assayed according to manufacturer instructions.

The components and concentration of the working solution were:

- Tris/Carbonate buffer-----pH 10.2 ± 0.2 mol/l
- p-Nitrophenyl phosphate-----16 mmol/l
- Magnesium chloride-----4 mmol/l

Estimation of ALP level was carried out in the Department of Biochemistry, under the guidance of an expert Biochemist by using spectrophotometric test which was carried out with the help of a Semi-autoanalyser using International Federation of Clinical Chemistry (IFCC) method on the basis of the adaptations. To which 950 µl of the reagent was mixed with the sample by using microcapillary pipette to make the total concentration of 1000 µl.

2.4. Formulation of 0.2% Thymoquinone gel

99% pure extract of Thymoquinone (Extract from Sigma Aldrich Company – Product No. 274666) was obtained in crystal form which was converted to powdered form using a mortar and pestle. Then all the ingredients were accurately weighed and a total bulk of 500 gm of gel was prepared. Base of the gel was prepared by 14 gm of carbopol mixed with 485 ml of hot water heated at a temperature of 80–90° C and stirred for 30 min to facilitate hydration. 1 gm of Thymoquinone was added to the mixture to attain a 0.2% concentration of Thymoquinone in the gel prepared and was stirred until homogenization. The pH of the gel was adjusted to 7.0 with 1 N Sodium hydroxide. Then the mixture was cooled at the room temperature (35 °C) and 2 drops of the Strawberry flavour were added to the mixture.

The drug was analyzed at 253 nm on UV–visible spectrophotometer for drug release estimation. The results showed that drug release from 0.2% Thymoquinone gel after 8 h was 95.01 ± 1.26 .

2.5. Local drug delivery

The prepared 0.2% Thymoquinone gel was loaded into a disposable insulin syringe with 26 gauge angulated blunt tip. The tip of the needle was slowly slid over the tooth surface till the base of the pocket, and then the gel was injected slowly till the pocket was overfilled. Excess gel was removed and the subjects were instructed not to drink, eat, brush and rinse for an hour. This procedure was repeated every week starting from baseline up to 4 weeks.

Group I sites were treated with scaling and root planing (SRP) along with subgingival application of 0.2% Thymoquinone Gel. Group II sites were treated with scaling and root planing only.

Intracrevicular application of 0.2% Thymoquinone Gel was done at baseline, 2, 3 and 4 weeks following SRP. All the selected sites were subjected to assessment of clinical parameters like Plaque Index (PI), Gingival Index (GI), Pocket Probing Depth (PPD), Relative Attachment Level

(RAL) and Biochemical Parameter like Alkaline Phosphatase (ALP) level in GCF tested at baseline and at 6 weeks post operatively.

2.6. Evaluation of parameters

The PI assessment was carried out according to the procedure followed by Vandekerckhove et al. (1998) and GI assessment was carried out using the procedure followed by Clark et al. (1987). Measurements of PPD and RAL were recorded using UNC-15 probe (Guentsch et al., 2008) and occlusal stent (Clark et al., 1987) respectively. ALP level was measured by collecting GCF samples from selected sites (Kunjappu et al., 2012). All GCF samples were collected in the forenoon (between 10 and 11 AM) to allow for the circadian variation seen in GCF volume (Bissada et al., 1967; Kunjappu et al., 2012). The parameters were assessed at baseline and at 6 weeks postoperatively.

Additionally an in vitro assessment of antibacterial efficacy of prepared gel was carried out on the strains of *Porphyromonas gingivalis* (ATCC 33277), *Aggregatibacter actinomycetemcomitans* (ATCC 29523) and *Prevotella intermedia* (ATCC 25611) under the guidance of an expert microbiologist which showed that all three strains were highly sensitive to the gel up to the dilution level of 10^{-9} .

2.7. Statistical analyses

The arithmetic mean and standard deviations were calculated for the requisite assessment intervals. For intra-group comparison ‘Wilcoxon Signed Rank Test’ was performed and non-parametric analysis of clinical parameters. ‘Independent t-test’ was performed for intra-group and inter-group comparison for changes in ALP levels in GCF and for comparison of inter-group variations in clinical parameters.

3. Results

The findings obtained from the test sites of Group I and Group II were subjected to comparison and assessment of clinical parameters i.e. PI, GI, PPD, RAL (Figs. 1 and 2) and biochemical parameter i.e. ALP levels in GCF at baseline and at 6 weeks interval. No adverse reactions or complications secondary to delivery of 0.2% Thymoquinone gel were observed. There was statistically highly significant reduction in PI, GI, PPD & gain in RAL in both the groups at 6 weeks from baseline (Table 1). Highly significant reduction in Gingival Crevicular Fluid Alkaline phosphatase (GCF-ALP) level was observed in both the groups at 6 weeks from baseline (Table 1). On comparison between Group I & Group II, former showed statistically significant reduction in PPD, GCF-ALP levels and gain in RAL but statistically not significant differences in PI and GI at 6 weeks (Table 2). On microbiological assessment 0.2% Thymoquinone gel was observed to be sensitive against *P. gingivalis*, *P. Intermedia* and *A. actinomycetemcomitans* (Table 3).

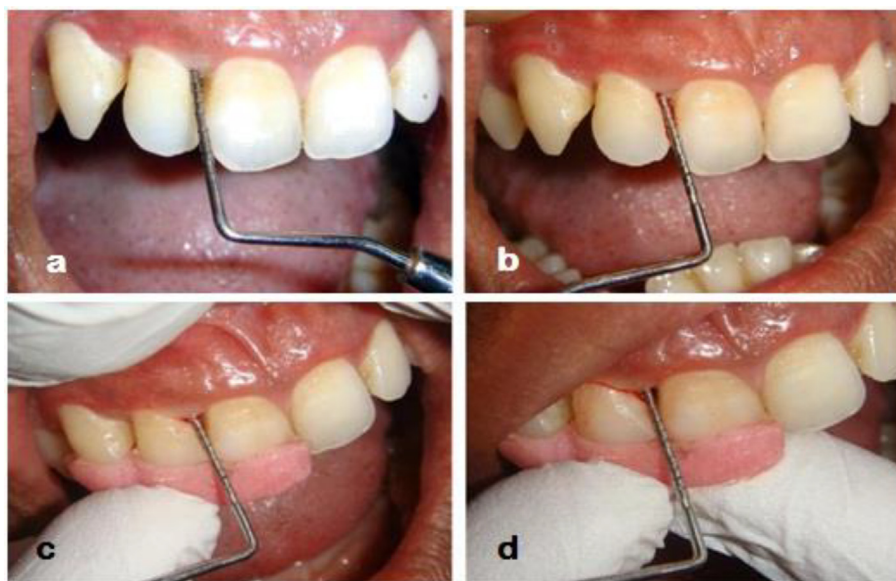


Fig. 1 (Group I) (a) Preoperative pocket probing depth, (b) postoperative pocket probing depth, (c) preoperative relative attachment level, (d) postoperative relative attachment level.

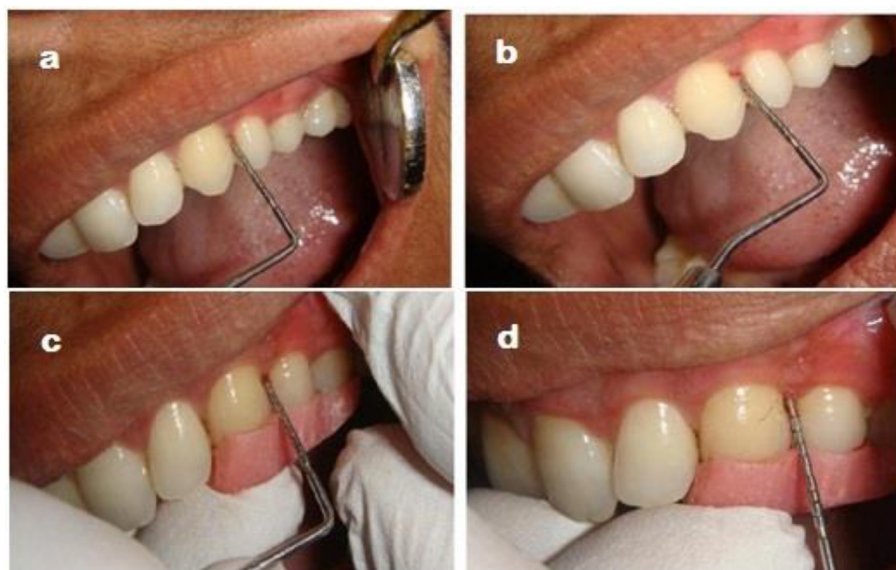


Fig. 2 (Group II) (a) Preoperative pocket probing depth, (b) postoperative pocket probing depth, (c) preoperative relative attachment level, (d) postoperative relative attachment level.

4. Discussion

The rationale of targeting localized areas of periodontal destruction have led to great interest in controlled release of drugs through local delivery systems to reduce the subgingival bacterial load left after SRP in periodontal pockets (Fiorellini and Paquette, 1992; Jones et al., 1994; Kornman, 1993; Needleman, 1991).

To overcome the adverse effects of antimicrobial/antibiotics, World Health Organization (WHO) advocated that the possibility of using natural products should be investigated,

such as plant extracts or herbs as an alternative. Plant extracts and herbs that have been used previously include *Salvadora persica* (Al-Bayaty et al., 2010), *Arnica montana* and *Hamamelis virginiana* which have shown to exhibit antibacterial activity against periodontopathic bacteria (Sultan et al., 2009; Vahabi et al., 2011).

Thymoquinone has been a source of debate for various researchers to have anti-inflammatory potential (Tekeoglu et al., 2007). Considering the potential properties and the capability of significantly inhibiting the expression of pro-inflammatory cytokines, thymoquinone may play a significant

Table 1 Mean and mean differences in clinical (plaque index, gingival index, pocket probing depth and relative attachment level) and biochemical (alkaline phosphatase enzyme level) parameters of Group I and Group II.

	Group I					Group II						
	No. of Sites	At baseline (preoperative)	At 6 weeks (postoperative)	Mean difference	Z	p value	No. of Sites	At baseline (preoperative)	At 6 weeks (postoperative)	Mean difference	Z	p value
PI	20	2.55 ± 0.510	1.20 ± 0.410	1.350 ± 0.587	-3.95	0.001*	20	2.55 ± 0.510	1.45 ± 0.510	1.100 ± 0.552	-3.97	0.001*
GI	20	2.45 ± 0.605	1.20 ± 0.410	1.250 ± 0.638	-3.85	0.001*	20	2.45 ± 0.605	1.25 ± 0.444	1.20 ± 0.615	-3.87	0.001*
PPD	20	5.25 ± 0.550	3.25 ± 0.716	2.000 ± 0.725	-4.06	0.001*	20	5.25 ± 0.550	3.80 ± 0.768	1.450 ± 0.686	-3.85	0.001*
RAL	20	9.05 ± 2.064	7.05 ± 2.038	2.00 ± 0.725	-4.06	0.001*	20	8.40 ± 2.280	6.95 ± 2.282	1.450 ± 0.686	-3.85	0.001*
ALP	20	350.450 ± 113.737	226.900 ± 79.566	123.55 ± 61.374	t	0.001*	20	386.200 ± 119.628	297.700 ± 102.517	88.5 ± 46.527	t	0.001*
					9.003						8.506	

p < 0.001.

* Highly significant.

role in preventing the initiation and progression of periodontitis (Ozdemir et al., 2012).

Observations by Ozdemir et al. (2012) have shown that gastric feeding of Thymoquinone in periodontitis induced rat models diminished alveolar bone resorption. Recent, clinical study by Al-Bayaty et al. (2013) advocated the use of periodontal chips as an adjunct containing thymoquinone for the treatment of chronic periodontitis which showed significant improvement in clinical parameters. Kouidhi et al. (2011) suggested that Thymoquinone could be used as a source of natural product as it possesses a selective antibacterial activity against oral bacteria with resistance-modifying activity.

Thymoquinone has a significant day and dose dependent antimicrobial activity against Gram-positive and Gram-negative bacteria (Islam et al., 2012) and its activity could be synergized by the use of antibiotics particularly in case of *S. aureus* (EmanHalawani, 2009). Mohany et al. (2012) and Nili-Ahmadabadi et al. (2011) reported significant decrease in ALP level when Thymoquinone was administered orally and intraperitoneally in rat models.

Thus, considering the above mentioned studies, and various properties of Thymoquinone, an attempt has been made through this study to evaluate the efficacy of 0.2% Thymoquinone gel in the treatment of chronic periodontitis based on assessment of various clinical and biochemical parameters (Antczak-Bouckoms et al., 1990; Ramfjord et al. 1968).

Patients were selected on the basis of inclusion and exclusion criteria by Guentsch et al. (2008) and Sastravaha et al. (2003).

In the present study, an in vitro assessment of antibacterial activity of 0.2% Thymoquinone gel was performed which demonstrated that *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were highly sensitive to 0.2% Thymoquinone gel up to a dilution level of 10^{-9} while *Prevotella intermedia* was highly sensitive up to a dilution level of 10^{-8} from which it was confirmed that all the three bacterial strains were highly sensitive against 0.2% Thymoquinone gel. Due to the complex nature of periodontal diseases here it is advocated that the drug efficiency should also be confirmed against more periodontal pathogens.

In this present study, GCF-ALP level estimation has been performed since it is a potential prognostic biomarker of periodontal disease activity.

Ishikawa and Cimasoni (1970) significantly correlated GCF ALP level to pocket depth. They observed that GCF ALP level was 3 times higher than that of serum ($r = 0.49$; $i < 0.05$).

However, further histological studies are required to gather information necessary to explain mechanism at different levels responsible for enhancing effects of the gel on periodontal healing. Further longitudinal investigation on larger sample size is required to assess its long term effectiveness as an adjunct to non-surgical treatment of periodontal pockets.

Hence, intracrevicular application of 0.2% Thymoquinone could be a beneficial adjunct to scaling and root planing in treatment of chronic periodontitis. Additionally investigations need to be carried out to evaluate whether or not Thymoquinone can be used subgingivally as an alternative to local antibiotics in order to control inflammation.

Table 2 Mean differences of various parameters comparison between Group I and Group II.

	Group I	Group II	Mean difference	t	p value
PI	1.350 ± 0.587	1.100 ± 0.552	0.250	1.387	0.174
GI	1.250 ± 0.638	1.200 ± 0.615	0.050	0.252	0.802
PPD	2.000 ± 0.725	1.450 ± 0.686	0.550	2.463	0.018*
RAL	2.000 ± 0.725	1.450 ± 0.686	0.550	2.463	0.018*
ALP	123.55 ± 61.374	88.5 ± 46.527	35.050	2.035	0.048

p < 0.05.

* Significant.

Table 3 Minimum inhibitory concentration results of 0.2% Thymoquinone gel against *Porphyromonasgingivalis* (Pg), *Aggregatibacteractinomycetemcomitans* (Aa) and *Prevotellaintermedia* (Pi).

Dilution Level	1	2	3	4	5	6	7	8	9	10
Pg	S	S	S	S	S	S	S	S	S	S
Aa	S	S	S	S	S	S	S	S	S	S
Pi	S	S	S	S	S	S	S	S	S	R

S-Sensitive, R-Resistant.

5. Conclusion

The overall observations of the present study were encouraging and showed promising results with significant variations in clinical and biochemical parameters. Further longitudinal studies including the histological observations need to be undertaken to further authenticate the effect of 0.2% Thymoquinone gel.

Conflict of interest

The authors declare that there are no conflict of interest.

Ethical statement

All the ethical concerns are taken into consideration before submitting the above mentioned manuscript.

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